

Effects of biopolymer-based active coatings on postharvest quality of okra pods in Tunisia

H. Aloui, N. Jguirim and K. Khwaldia^a

Laboratoire des Substances Naturelles (LSN, LR10 INRAP02), Institut National de Recherche et d'Analyse Physico-chimique (INRAP), Pôle Technologique de Sidi Thabet, 2020 Sidi Thabet, Tunisia

Summary

Introduction – Fresh okra represents an important source of nutrients for people in Tunisia. However, being climacteric, this crop fruit has a brief shelf life. The objective of this study was to assess the ability of sodium alginate (NaAlg)-based coatings incorporating bergamot or bitter orange essential oils (EO) to enhance the fruit quality and shelf life of okra (*Abelmoschus esculentus*) pods and retard fungal contamination caused by *Cladosporium oxysporum*. **Materials and methods** – The effects of the different treatments on physical characteristics of okra pods var. Marsaouia were measured over 12 days of cold storage in terms of weight loss, firmness and respiration rate of the fruit, while their efficacy in lowering fungal contamination was assessed over 8 days of storage at 25 °C. Likewise, sensory analysis was carried out to investigate the effect of the developed coatings on sensory attributes of fresh okra pods. **Results and discussion** – The applied coatings were able to lower respiration of okra pods and decrease weight and firmness losses by more than 36% and 18%, respectively, at the end of the storage period. The inclusion of citrus essential oil into NaAlg-based coating preserved the sensory attributes of okra pods, delayed the disease onset date and reduced by two-third the number of decayed pods compared with uncoated samples and those treated with neat NaAlg. **Conclusion** – NaAlg coatings amended with citrus oils show promising efficacy as substitutes to synthetic fungicides in the postharvest management of okra pods produced in Tunisia.

Keywords

Tunisia, okra, *Abelmoschus esculentus*, sodium alginate, postharvest management, fruit quality, sensorial analysis

Résumé

Effets d'enrobages actifs à base de biopolymères sur la qualité post-récolte des gousses de gombo en Tunisie.

Introduction – Le gombo frais représente une source importante de nutriments pour la population tunisienne. Cependant, les fruits de cette espèce étant climactériques, ils présentent une courte durée de conservation. L'objectif de cette étude était d'évaluer la capacité des enrobages à base d'alginate de sodium

Significance of this study

What is already known on this subject?

- Fresh okra represents an important source of nutrients for people in Tunisia. Being climacteric, this crop fruit has a short shelf life.

What are the new findings?

- Alginate coatings amended with citrus oils on 'Marsaouia' okra pods were effective in extending their shelf life and reducing fungal decay.

What is the expected impact on horticulture?

- Edible coatings offer a viable alternative to the use of present day synthetic fungicides in the postharvest management of okra pods in Tunisia.

(NaAlg) avec ou sans huile essentielle de bergamote ou d'orange amère à améliorer la qualité et la durée de conservation des gousses d'okra (*Abelmoschus esculentus* (L.) Moench) et à retarder la contamination fongique causée par *Cladosporium oxysporum*. **Matériel et méthodes** – Les effets des différents traitements sur les caractéristiques physiques des gousses de gombo var. Marsaouia ont été mesurés sur 12 jours d'entreposage frigorifique en termes de perte de poids, de fermeté et de respiration des fruits, tandis que leur efficacité à réduire la contamination fongique a été évaluée sur 8 jours de stockage à 25 °C. De même, une analyse sensorielle a été réalisée pour étudier l'effet des enrobages sur les attributs sensoriels des gousses fraîches de gombo. **Résultats et discussion** – Les enrobages testés ont permis réduire le taux de respiration des gousses de gombo et de réduire les pertes de poids et de fermeté de plus de 36% et 18%, respectivement, à la fin de la période de stockage. L'inclusion des huiles essentielles d'agrumes dans les enrobages à base de NaAlg a préservé les attributs sensoriels des gousses de gombo, retardé la date d'apparition de la maladie et réduit des deux-tiers le nombre de gousses infectées par rapport aux échantillons non enrobés et ceux traités avec l'alginate pure. **Conclusion** – Les enrobages à base d'alginate incorporant les huiles essentielles d'agrumes présentent une efficacité prometteuse en tant que substituts aux fongicides synthétiques dans la gestion post-récolte des gousses de gombo produites en Tunisie.

^a Corresponding author: khaoula_khwaldia@yahoo.fr

Mots-clés

Tunisie, gombo, *Abelmoschus esculentus*, alginate de sodium, gestion post-récolte, qualité des fruits, analyse sensorielle

Introduction

Okra (*Abelmoschus esculentus* (L.) Moench), also referred to as lady's finger, is among the most consumed commodities in Tunisia. It is known for its high content of nutrients (Arapitsas, 2008) as well as its medicinal potential against inflammation, gastric irritation and colon cancer (Babarinde and Fabunmi, 2009). However, being climacteric, this crop has a brief shelf life (Finger *et al.*, 2008).

Moreover, during handling, transportation and storage, okra may be infected by various fungi including *Cladosporium oxysporum*, *Aspergillus flavus*, *A. niger*, *Alternaria alternata*, *Curvularia lunata*, *Rhizopus nigricans*, *Stachybotrys atra*, *Chaetomium globosum*, *Rhizoctonia bataticola* (Sharma *et al.*, 2013). These postharvest contaminations may reduce the storage period and commercial shelf life of fresh okra and result in economic loss, especially for exporting countries. Chlorine solution has been widely used for reducing fungal decay in fresh okra pods (Salunkhe and Desai, 1984). However, problems regarding the toxicity of chemical residues have prompted an investigation of potentially less harmful methods. In this sense, storage at low temperature has been proposed as an alternative tool to replace chemical treatments, owing to its ability to delay fruit senescence (Dhall *et al.*, 2014). However, this treatment has received very little attention, as its beneficial effects are often limited by the development of chilling injury-associated disorders, including skin browning, translucency or pitting (Boontongto *et al.*, 2007).

Recently, there has been an increasing interest in the use of natural compounds such as essential oils (EO) as promising alternatives to chemical-based preservatives for maintaining fruit quality and reducing postharvest fungal decay (Kalemba and Kunicka, 2003). Among a wide variety of EO, those from citrus fruit have attracted more attention as antifungal agents owing to their strong inhibitory effect against molds commonly associated with food spoilage, including species of *Penicillium*, *Aspergillus* and *Cladosporium* (Dimić *et al.*, 2014). The high antifungal potential of citrus EO was ascribed to the presence of bioactive components such as D-limonene, linalool or citral as well as to the ability of their amphiphilic phenolic compounds to interact with both polar and aliphatic sides of fungal membranes (Veldhuizen *et al.*, 2006).

Although most of the EO are generally recognized as safe (GRAS), their use as food preservatives, is often restricted mainly because of their high volatility, vigorous aroma and flavor and potential toxicity. Recently, the incorporation of EO into edible coatings has been investigated as an effective approach to solve some of these problems by entrapping active molecules, lowering their diffusion rate and maintaining high concentrations of them on the surface of the product (Gyawali and Ibrahim, 2014). Moreover, edible coatings can extend the shelf life of fresh fruits and vegetables by acting as a semi-permeable barrier able to reduce weight loss, gas exchange, respiration and oxidative reaction rates (Hoa *et al.*, 2002; Shao *et al.*, 2012). Over the last few years, sodium alginate (NaAlg), derived from marine brown algae (*Phaeophyceae*), has been investigated as a promising coat-

ing material owing to its excellent film forming properties and its selective permeability to O₂ and CO₂ (Oms-Oliu *et al.*, 2008). Moreover, NaAlg-based coatings have been shown to act as an effective matrix for the entrapment of antimicrobial compounds which may improve their ability to protect fresh fruits and vegetables by limiting microbial growth on their surface (Rojas-Graù *et al.*, 2009). Recently, Aloui *et al.* (2014a) reported the beneficial effect of NaAlg coatings enriched with grapefruit EO in preserving the postharvest quality of table grapes and reducing the growth of *Penicillium digitatum*. In another study, Azarakhsh *et al.* (2014) revealed that NaAlg-lemongrass EO composite coatings were effective in reducing yeast and mold count and maintaining quality attributes of fresh-cut pineapple.

Although many studies have demonstrated the efficacy of edible coatings incorporating EO in reducing fungal decay and delaying ripening of several commodities (Aloui *et al.*, 2014a, 2014b; Perdones *et al.*, 2012; Sánchez-González *et al.*, 2011), there has been no research on their use for preserving fresh okra pods.

Therefore, the aim of this study was to evaluate the effectiveness of NaAlg coatings incorporating citrus EO derived from two different species namely bergamot (*Citrus bergamia* Risso) and bitter orange (*Citrus aurantium* L.) at preserving quality attributes of fresh okra pods and controlling postharvest fungal spoilage caused by *Cladosporium oxysporum*.

Materials and methods**Raw materials**

Fresh okra pods (*Abelmoschus esculentus* var. Marsaouia) were purchased from a local wholesale distributor in Tunis (Tunisia) and immediately transported to the laboratory in polystyrene boxes at ambient conditions of temperature and humidity (18 °C and 75% RH). Pods of uniform size, shape and color and without any signs of physical damage and/or disease symptoms were selected and washed in 10 mL L⁻¹ sodium hypochlorite solution for 2 min, before being washed and air-dried at room temperature for 2 h. Coating experiments were carried out on the same day.

Food-grade NaAlg (molecular weight ~80,000 Da, CAS Number 9005-38-3) used as a coating material in this study was purchased from Sigma-Aldrich (Steinheim, Germany). Bergamot and bitter orange EO were kindly supplied by the University of Catania (Italy). The composition and the main physicochemical properties of these two citrus oils are presented in Table 1.

TABLE 1. Refraction index, density and major chemical volatiles compounds of bergamot and bitter orange essential oils. The upper and lower limits of each interval are determined based on 5 measurements.

Citrus essential oils	Refraction index	Density (g mL ⁻¹)	Major volatile compounds
Bergamot	1.460–1.480	0.872–0.882	Limonene (40%) Linalool (8%) Citral (0.580%)
Bitter orange	1.472–1.476	0.840–0.860	Limonene (93%) Linalool (0.270%) Citral (0.120%)

Preparation and application of coating solutions

A 2% (w/v) NaAlg coating solution was prepared by dissolving 2 g NaAlg in 100 mL distilled water heated at 70 °C and stirred overnight at room temperature for complete dissolution. Composite solutions were prepared by adding a 2% (v/v) concentration of either bergamot or bitter orange EO to the prepared NaAlg solutions. This concentration was defined as the minimum effective concentration for inhibiting *Cladosporium oxysporum* conidial germination based on our preliminary assays (data not shown). The mixtures were then emulsified at 13,500 rpm for 4 min, using an Ultra-Turrax T25 (IKA, Labortechnik GmbH, Munich, Germany) before being degassed at room temperature using a vacuum pump to remove air bubbles.

Before coating application, selected okra pods were randomly assigned to four treatment groups, named Control: uncoated okra pods; NaAlg: okra pods coated with pure NaAlg; NaAlg-bergamot EO: okra pods coated with NaAlg enriched with 2% bergamot EO; NaAlg-bitter orange EO: okra pods coated with NaAlg enriched with 2% bitter orange EO. Subsequently, okra pods were completely immersed in the different NaAlg coating solutions for 1 min to achieve uniform surface coatings, before being drained and dried at room temperature for 2 h. Finally all samples were stored in perforated PET trays at 4 °C and 85–90% relative humidity (RH) for 12 days.

Okra pods characterization

Forty-five okra pods for each treatment and sampling date were analyzed at intervals of 3 days over a storage period of 12 days to evaluate the effect of the applied coatings on their quality attributes. All the analyses were performed in triplicate.

Weight loss

To determine weight loss, the same okra pods were weighed before storage and at the end of each storage period. Weight loss was expressed as the percentage loss of the initial total weight.

Fruit firmness

Texture analysis was performed according to Aloui *et al.* (2014a) by measuring force at break (N) using a computer-controlled Universal Testing Machine (Instron, Model 3345, USA) equipped with a 50 kg load cell. Samples were 50% compressed at a constant speed of 2 mm s⁻¹, using a 6-mm diameter round tipped puncture probe.

Respiration rate

Respiration of coated and uncoated okra pods was measured using a closed system as described by Sánchez-González *et al.* (2011). Samples were weighed (approximately 100 g for each treatment and sampling date) and placed in 0.580-L hermetic glass jars closed with air-tight screw caps with a rubber septum to allow sampling gas in the headspace. The jars were immediately stored at 4 °C. O₂ and CO₂ contents were measured every 30 min by means of a needle connected to a head space gas analyzer (Checkmate 9900, PBI Dansensor, Denmark). Three replicates were carried out for each treatment.

Respiration rates in terms of O₂ consumption and CO₂ generation (mL kg⁻¹ h⁻¹) were determined from the slopes of O₂ and CO₂ concentrations versus time, as described by Fonseca *et al.* (2002).

Fungal decay

Fungus isolation and culture conditions

Cladosporium oxysporum was isolated from diseased okra pods. Small portions of symptomatic tissue were disinfected with 1% sodium hypochlorite for 1 min and placed at the center of a Petri plate containing potato dextrose agar medium (PDA; Biokar Diagnostics, Beauvais, France). After 7 days of incubation at 25 °C, growing fungal colonies were sub-cultured and identified based on morphological characters such the length of their conidiophores and the size and shape of the conidia.

Okra decay

Thirty okra pods for each treatment were washed in 10 mL L⁻¹ sodium hypochlorite solution and superficially wounded using a stainless steel rod with a probe tip 1 mm wide and 2 mm in length. The injured pods were then dipped in a conidial suspension of *C. oxysporum* at a concentration of 10⁵ conidia mL⁻¹ for 1 min, before being dried at room temperature for 2 h. Inoculated fruits were coated by immersion for 1 min in the different NaAlg solutions and air-dried at room temperature.

Thirty uncoated okra pods were used as a control. After complete drying, all samples were stored at simulated marketing conditions (25 °C, 85–90% RH) for 8 days and the decay percentage of coated and uncoated pods, expressed as the number of decayed pods out of the total number of pods per treatment, was daily evaluated. Each experiment was conducted twice.

Sensory analysis

Sensory evaluation was carried out after 2 days of cold storage by 30 non-trained panelists aged between 21 and 40 years old. This time-frame was chosen based on the previous studies reporting that the largest differences related to coated foods evaluation are often observed at the beginning of the storage period (Del-Valle *et al.*, 2005; Synowiec *et al.*, 2014). Attributes which consisted of adhesiveness, firmness, color, citrus odor and brightness were evaluated using a 9-point intensity scale where 1 indicated extreme dislike and 9 indicated extreme like. Evaluations were conducted in individual booths under white illumination at room temperature. The order of presentation was randomized between judges and sessions.

Statistical analysis

Statistical analysis was performed using Statgraphics® Plus 5.1 (Manugistics Inc., Rockville, MD, USA). Differences between means were determined by Fisher's protected least significant difference test (LSD, $P < 0.05$) performed after a multifactor analysis of variance (ANOVA). Data on fungal decay have been subjected to the arcsine transformation before applying the ANOVA in order to assure the homogeneity of variances.

Results and discussion

Okra pods characterization

Weight loss

As shown in Figure 1 all samples underwent a gradual loss of weight during storage ($P < 0.05$) due to water loss occurring during respiration, transpiration and evaporation processes. Overall, NaAlg coatings significantly reduced

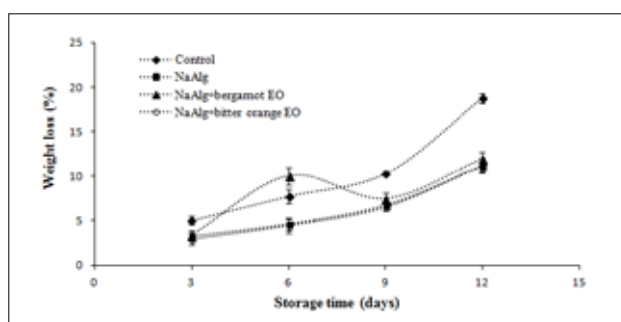


FIGURE 1. Weight loss of uncoated (◆) and coated okra pods (■ NaAlg; ▲ NaAlg + bergamot EO; ○ NaAlg + bitter orange EO) stored at 4 °C for 12 days. Each value represents the mean and LSD interval of three randomly selected replicates from 45 fruits per treatment. NaAlg: sodium alginate; EO: essential oil.

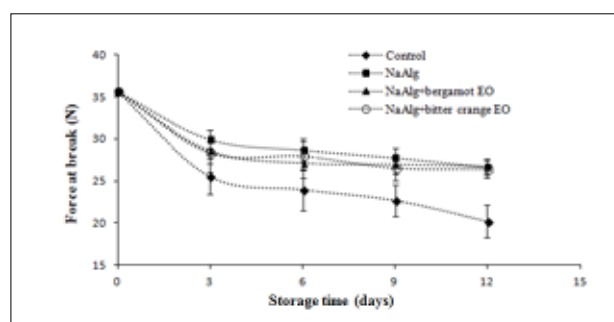


FIGURE 2. Force at break of uncoated (◆) and coated okra pods (■ NaAlg; ▲ NaAlg + bergamot EO; ○ NaAlg + bitter orange EO) stored at 4 °C for 12 days. Each value represents the mean and LSD interval of three randomly selected replicates from 45 fruits per treatment. NaAlg: sodium alginate; EO: essential oil.

weight loss of coated okra pods compared with the control ($P < 0.05$), highlighting the ability of these coatings to act as a protective barrier against water evaporation, gas exchange and nutrient loss. However, no significant effect on the weight loss of coated okra pods was observed upon the incorporation of bergamot and bitter orange EO into NaAlg coating formulations. These findings differ from those obtained previously by Aloui *et al.* (2014a), who reported an increase in water loss of coated grapes when grapefruit EO was incorporated into NaAlg matrix. Conversely, Sánchez-González *et al.* (2011) noticed a significant reduction in weight loss of grapes when bergamot EO was added to either chitosan or hydroxypropyl methylcellulose coating formulations.

Regardless of coating treatment, the applied coatings were able to reduce weight loss of okra pods in the range of 36–41% at day 12, compared with the control ($P < 0.05$). Our results are consistent with previous studies reporting the efficacy of NaAlg coatings with or without citrus EO against weight loss of grapes (Aloui *et al.*, 2014a) and cut pineapples (Azarakhsh *et al.*, 2014).

Firmness

Firmness decreased gradually throughout the storage time for both treated and untreated okra pods (Figure 2) due to tissue senescence and loss of membrane integrity resulting from the hydrolysis of cellulose and hemicellulose

as well as the depolymerization of pectin and starch (Xiao *et al.*, 2010). Overall, coated pods presented a significantly ($P < 0.05$) higher firmness than control samples which lost more than 43% of their initial firmness within 12 days of storage ($P < 0.05$). However no significant effect of citrus EO incorporation was found in terms of mechanical resistance of coated samples ($P < 0.05$). Regardless of the coating type, firmness loss reduction in coated okra pods was around 18% at day 12, compared with the control ($P < 0.05$), underlining the high potential of NaAlg coatings to delay softening process as previously reported by several authors (Aloui *et al.*, 2015; Guerreiro *et al.*, 2015). In contrast to our results, Aloui *et al.* (2014a) and Azarakhsh *et al.* (2014) reported a significant decrease in the firmness of coated grapes and cut pineapples respectively, when lemongrass and grapefruit EO were added to NaAlg coatings. This behavior was ascribed to the weakening effect exerted by citrus EO on polymer resistance as well as to the action of their active compounds on fruit cell tissue which possibly undergo structural changes that directly affect the metabolic patterns of the fruit and lead to the loss of its firmness (Perdones *et al.*, 2012; Xiao *et al.*, 2010).

Respiration rate

The effects of the different NaAlg coatings on the respiration rate of fresh okra pods were evaluated through O_2 con-

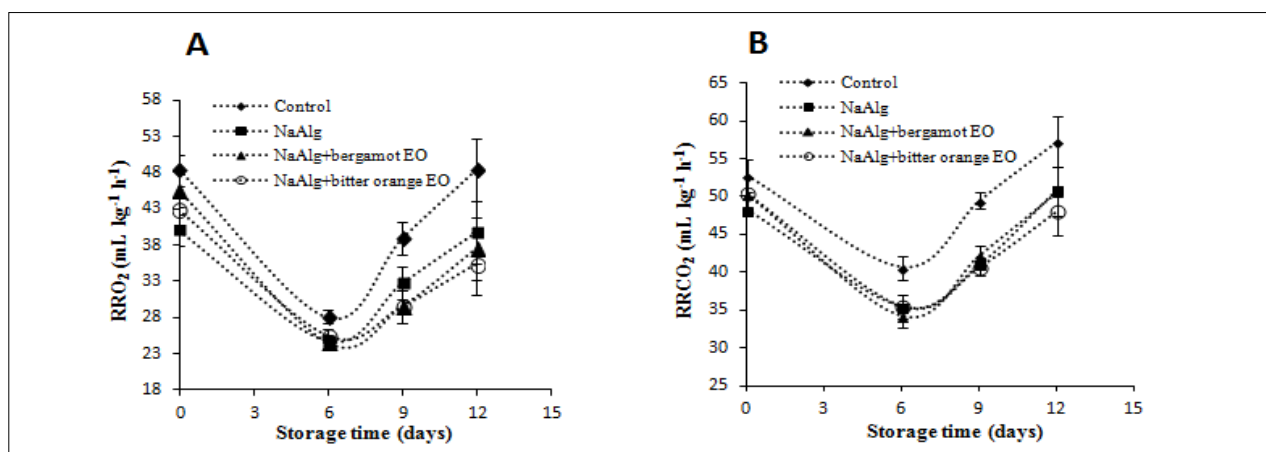


FIGURE 3. Respiration rate in terms of O_2 (a) and CO_2 (b) as a function of storage time and coating formulation. ◆ Control; ■ NaAlg; ▲ NaAlg + bergamot EO; ○ NaAlg + bitter orange EO. Each value represents the mean and LSD interval of three randomly selected replicates from 45 fruits per treatment. NaAlg: sodium alginate; EO: essential oil.

sumption and CO₂ generation (Figure 3). The respiration rate of both coated and uncoated samples showed a significant decrease within the first 6 days ($P < 0.05$) and then increased gradually during the remaining days ($P < 0.05$) exhibiting a respiration pattern of climacteric fruit. Such increase in the respiration rate could be explained by an increase in the fruit metabolic activity related to tissue senescence and cell breakdown. Overall a significant delay in both O₂ consumption and CO₂ production was observed when NaAlg coatings were applied on okra pods ($P < 0.05$). These coatings can modify the internal atmosphere of okra pods by decreasing the interchange of CO₂ and O₂ between the environment and coated fruit, owing to their barrier effects against O₂ and CO₂ diffusion (González-Aguilar *et al.*, 2010). Statistical analysis revealed that the incorporation of either bergamot or bitter orange EO into NaAlg coatings did not significantly modify the respiration rate of coated okra pods ($P < 0.05$). A similar trend was reported by Sánchez-González *et al.* (2011) when evaluating the effect of hydroxypropyl-methylcellulose coatings incorporated with bergamot EO on the respiration pattern of coated grapes. Conversely, Azarakhsh *et al.* (2014) and Perdonés *et al.* (2012) noticed a significant decrease in the respiration rate of cut pineapples and strawberries when either lemongrass or bergamot EO were added respectively to NaAlg and chitosan coatings. Many factors such as the type and shape of the raw material, the stage of maturity as well as the nature of interactions between the fruit and the coating material may strongly affect the respiration rate of coated fruits and result in different gas barrier properties.

Regardless of coating formulation, O₂ consumption reduction in coated okra pods was about 26% at day 12, compared to uncoated control samples ($P < 0.05$), whereas that of CO₂ production was in the range of 11–16% ($P < 0.05$). Previous studies have reported the efficacy of polysaccharide based coatings in reducing respiration rate of a wide range of fruits including cherry tomato (Fagundes *et al.*, 2014) and strawberry (Velickova *et al.*, 2013).

Okra decay

Figure 4 shows the decay percentage of inoculated okra pods as a function of treatment and storage time. As depicted in this figure, decay incidence increased with the increasing

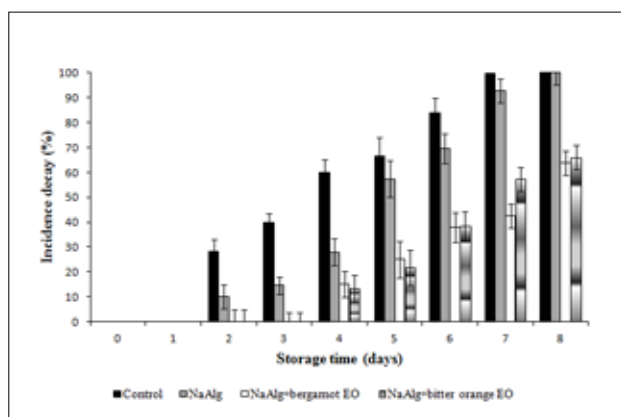


FIGURE 4. Effect of coatings formulated with sodium alginate either alone (■ NaAlg) or combined with bergamot (▲) or bitter orange (○) EO on fungal decay of artificially inoculated okra pods stored at 25 °C. Each value represents the mean and LSD interval of two randomly selected replicates from 60 fruits per treatment. NaAlg: sodium alginate; EO: essential oil.

of the storage time and was significantly higher for uncoated okra pods and those treated with pure NaAlg, as opposed to those dipped into NaAlg-EO emulsions ($P < 0.05$). A significant delay in the infection level by *C. oxysporum* was noticed for samples treated with pure NaAlg coatings which were able to reduce fungal decay incidence by more than 14% after 6 days of storage ($P < 0.05$). Such behavior, could be explained by the ability of the applied coatings to act as effective barriers against gaseous exchange by decreasing oxygen diffusion and favoring CO₂ accumulation in the atmosphere inside the fruit. In fact, decreasing interchange of CO₂ and O₂ between the environment and coated fruit may affect the growth of molds as they are strictly aerobic and very sensitive to CO₂ (Soliva-Fortuny *et al.*, 2004). This result was confirmed by our data on respiration rate (Figure 3).

This behavior is in accordance with the results of Aloui *et al.* (2014a, 2015) who reported the ability of pure NaAlg coatings to delay the growth of *Penicillium digitatum* in both inoculated grapes and oranges. NaAlg coatings enriched with citrus EO were effective at delaying the onset of disease symptoms up to the third day of storage (0% disease incidence) and reducing the growth of *C. oxysporum* in okra pods by more than 33% ($P < 0.05$) compared with the control and pure NaAlg coatings (Figure 4) at the end of the storage period. This result seems to be related to the high antifungal potential of citrus oils previously demonstrated against several postharvest molds including *C. oxysporum* (Veldhuizen *et al.*, 2006).

Similarly, Aloui *et al.* (2014a) reported a reduction by more than 50% in the growth of *Aspergillus flavus* when adding bergamot or bitter orange oils to NaAlg formulations, applied as coating materials to inoculated dates. Likewise, a significant reduction in the growth of *Botrytis cinerea* was noticed in inoculated strawberries when lemon EO was added to chitosan based coatings (Perdonés *et al.*, 2012). The high antifungal potential of citrus EO seems to be related to the presence of different bioactive components such as D-limonene, linalool or citral as well as the ability of their amphiphilic phenolic compounds to interact with both polar and aliphatic sides of fungal membranes (Gyawali and Ibrahim, 2014).

Sensory analysis

Overall, samples treated with NaAlg either alone or combined with citrus EO showed a good adhesiveness between the applied coatings and the okra skin (Table 2). However, the perception of coating adhesiveness was not significantly affected by coating type ($P < 0.05$).

On the other hand, judges evaluated samples treated with NaAlg coatings carrying bergamot or bitter orange EO as less glossy than control fruits and those treated with pure NaAlg ($P < 0.05$). Likewise, a significant decrease in color intensity of treated okra pods was observed as a result of citrus oil addition to NaAlg formulations ($P < 0.05$). During film drying step, oil droplets may aggregate near to the film surface, limiting light absorption through coated fruits and thus affecting, their glossiness and color intensity (Pastor *et al.*, 2011). Similar effects on glossiness and color intensity of coated dates were observed by Aloui *et al.* (2014) when the same citrus EO were added to either chitosan or Locust Bean Gum coating formulations. Although significant differences in firmness between the control and the different coated okra pods were noticed by the assessors ($P < 0.05$), no significant effect of citrus EO incorporation was found in terms of mechanical properties, which is in line with the instrumen-

TABLE 2. Effect of NaAlg coatings on the sensory characteristics of fresh okra pods after two days of storage at 4 °C. Data are mean values rated by 30 non-trained panel assessors aged between 21 and 40 years old. NaAlg: sodium alginate; EO: essential oil.

Attributes	Control	NaAlg	NaAlg + bergamot EO	NaAlg + bitter orange EO
Adhesiveness	4.60 ^a	6.75 ^b	6.93 ^b	6.84 ^b
Gloss	7.32 ^a	6.41 ^a	4.95 ^b	4.96 ^b
Color	6.35 ^a	5.38 ^{ab}	3.78 ^b	4.02 ^b
Firmness	5.44 ^a	7.54 ^b	6.89 ^b	7.11 ^b
Citrus odor	3.03 ^a	3.43 ^a	6.05 ^b	6.20 ^b

^{a-b} different superscripts within a file indicate significant differences among treatments using the LSD test at $P < 0.05$.

tal determinations described above (Figure 2). On the other hand, the sensory panel recorded a relatively high intensity of citrus aroma in okra pods treated with NaAlg formulations incorporated with bergamot or bitter orange EO.

Likewise, a high intensity of citrus odor was revealed in Tunisian date fruits when the same citrus oils were added to both chitosan and Locust Bean Gum coatings (Aloui et al., 2014b). Such high intensity of citrus aroma may affect the sensory characteristics of okra, as a consequence, a test of preference for overall acceptance evaluation would be the object of further sensory investigations.

Conclusion

The effects of NaAlg based-coatings amended with citrus essential oils on physical characteristics and fungal contamination of okra pods were studied in Tunisia. Regardless of the treatment type, the applied coatings are able to decrease significantly weight and firmness losses at the end of the storage period. NaAlg-based active coatings delay the disease onset date and decrease the number of decayed pods compared with uncoated samples and those treated with pure NaAlg. Our results underline the effectiveness of the designed active coatings on 'Marsaouia' okra pods in extending their shelf life and reducing fungal decay caused by *Cladosporium oxysporum*. Further studies are still needed to obtain a good compromise between the antimicrobial effectiveness of essential oils and their sensory impact.

Acknowledgments

The authors are grateful to Dr. Cristina Restuccia from the Dipartimento di Agricoltura, Alimentazione e Ambiente (Di3A), University of Catania, Italy, for kindly providing the citrus essential oils. The Tunisian Ministry of Higher Education and Scientific Research is gratefully acknowledged for its financial support.

References

Aloui, H., Khwaldia, K., Sánchez-González, L., Muneret, L., Jeandel, C., Hamdi, M., and Desobry, S. (2014a). Alginate coatings containing grapefruit essential oil or grapefruit seed extract for grapes preservation. *Int. J. Food Sci. Technol.* *49*, 952–959. <https://doi.org/10.1111/ijfs.12387>.

Aloui, H., Khwaldia, K., Licciardello, F., Mazzaglia, A., Muratore, G., Hamdi, M., and Restuccia, C. (2014b). Efficacy of the combined application of chitosan and Locust Bean Gum with different citrus essential oils to control postharvest spoilage caused by *Aspergillus flavus* in dates. *Int. J. Food Microbiol.* *170*, 21–28. <https://doi.org/10.1016/j.ijfoodmicro.2013.10.017>.

Aloui, H., Licciardello, F., Khwaldia, K., Hamdi, M., and Restuccia, C. (2015). Physical properties and antifungal activity of bioactive films containing *Wickerhamomyces anomalus* killer yeast and their application for preservation of oranges and control of postharvest green mould caused by *Penicillium digitatum*. *Int. J. Food Microbiol.* *200*, 22–30. <https://doi.org/10.1016/j.ijfoodmicro.2015.01.015>.

Arapitsas, P. (2008). Identification and quantification of polyphenolic compounds from okra seeds and skins. *Food Chem.* *110*, 1041–1045. <https://doi.org/10.1016/j.foodchem.2008.03.014>.

Azarakhsh, N., Osman, A., Ghazali, H.M., Tan, C.P., and Adzahan, N.M. (2014). Lemongrass essential oil incorporated into alginate-based edible coating for shelf-life extension and quality retention of fresh-cut pineapple. *Postharvest Biol. Technol.* *88*, 1–7. <https://doi.org/10.1016/j.postharvbio.2013.09.004>.

Babarinde, G.O., and Fabunmi, O.A. (2009). Effects of packaging materials and storage temperature on quality of fresh okra (*Abelmoschus esculentus*) fruit. *Agricultura Trop. Subtrop.* *42*, 151–156.

Boontongto, N., Srilaong, V., Uthairatanakij, A., Wongs-Aree, C., and Aryasuk, K. (2007). Effect of methyl jasmonate on chilling injury of okra pod. *Acta Hort.* *746*, 323–327. <https://doi.org/10.17660/ActaHortic.2007.746.37>.

Del-Valle, V., Hernández-Muñoz, P., Guarda, A., and Galotto, M.J. (2005). Development of a cactus-mucilage edible coating (*Opuntia ficus indica*) and its application to extend strawberry (*Fragaria ananassa*) shelf-life. *Food Chem.* *91*, 751–756. <https://doi.org/10.1016/j.foodchem.2004.07.002>.

Dhall, R.K., Sharma, S.R., and Mahajan, B.V.C. (2014). Development of post-harvest protocol of okra for export marketing. *J. Food Sci. Tech.* *51*, 1622–1625. <https://doi.org/10.1007/s13197-012-0669-0>.

Dimić, G., Kocić-Tanackov, S., Mojović, L., and Pejin, J. (2014). Antifungal activity of lemon essential oil, coriander and cinnamon extracts on foodborne molds in direct contact and the vapor phase. *J. Food Process. Pres.*, p. 1–10.

Fagundes, C., Palou, L., Monteiro, A.R., and Pérez-Gago, M.B. (2014). Effect of antifungal hydroxypropyl methylcellulose-beeswax edible coatings on gray mold development and quality attributes of cold-stored cherry tomato fruit. *Postharvest Biol. Technol.* *92*, 1–8. <https://doi.org/10.1016/j.postharvbio.2014.01.006>.

Finger, F.L., Della-Justina, M.E., Casali, V.W.D., and Puiatti, M. (2008). Temperature and modified atmosphere affect the quality of okra. *Sci. Agric. (Piracicaba Braz.)* *65*, 360–364. <https://doi.org/10.1590/S0103-90162008000400006>.

Fonseca, S.C., Oliveira, F.A.R., Jeffrey, K., and Brecha, J.K. (2002). Modelling respiration rate of fresh fruits and vegetables for modified atmosphere packages: a review. *J. Food Eng.* *52*, 99–119. [https://doi.org/10.1016/S0260-8774\(01\)00106-6](https://doi.org/10.1016/S0260-8774(01)00106-6).

- González-Aguilar, G.A., Ayala-Zavala, G.F., Olivas, G.I., de la Rosa, L.A., and Alvarez-Parrilla, E. (2010). Preserving quality of fresh-cut products using safe technologies. *J. Consum. Prot. Food Saf.* *5*, 65–72. <https://doi.org/10.1007/s00003-009-0315-6>.
- Guerreiro, A.C., Gago, C.M.L., Faleiro, M.L., Miguela, M.G.C., and Antunes, M.D.C. (2015). The effect of alginate-based edible coatings enriched with essential oils constituents on *Arbutus unedo* L. fresh fruit storage. *Postharvest Biol. Technol.* *100*, 226–233. <https://doi.org/10.1016/j.postharvbio.2014.09.002>.
- Gyawali, R., and Ibrahim, S.A. (2014). Natural products as antimicrobial agents: a review. *Food Control* *46*, 412–429. <https://doi.org/10.1016/j.foodcont.2014.05.047>.
- Hoa, T.T., Ducamp, M.N., Lebrun, M., and Baldwin, E.A. (2002). Effect of different coating treatments on the quality of mango fruit. *J. Food Qual.* *25*, 471–486. <https://doi.org/10.1111/j.1745-4557.2002.tb01041.x>.
- Kalemba, D., and Kunicka, A. (2003). Antibacterial and antifungal properties of essential oils. *Curr. Med. Chem.* *10*, 813–829. <https://doi.org/10.2174/0929867033457719>.
- Oms-Oliu, G., Soliva-Fortuny, R., and Martín-Belloso, O. (2008). Using polysaccharide-based edible coatings to enhance quality and antioxidant properties of fresh-cut melon. *Food Sci. Technol.* *41*, 1862–1870. <https://doi.org/10.1016/j.lwt.2008.01.007>.
- Pastor, C., Sánchez-González, L., Marcilla, A., Chiralt, A., Chafer, M., and González-Martínez, C. (2011). Quality and safety of table grapes coated with hydroxypropylmethylcellulose edible coatings containing propolis extract. *Postharvest Biol. Technol.* *60*, 64–70. <https://doi.org/10.1016/j.postharvbio.2010.11.003>.
- Perdones, A., Sánchez-González, L., Chiralt, A., and Vargas, M. (2012). Effect of chitosan-lemon essential oil coatings on storage-keeping quality of strawberry. *Postharvest Biol. Technol.* *70*, 32–41. <https://doi.org/10.1016/j.postharvbio.2012.04.002>.
- Rojas-Graù, M.A., Oms-Oliu, G., Soliva-Fortuny, R., and Martín-Belloso, O. (2009). The use of packaging techniques to maintain freshness in fresh-cut fruits and vegetables. *Int. J. Food Sci. Technol.* *44*, 875–889. <https://doi.org/10.1111/j.1365-2621.2009.01911.x>.
- Salunkhe, D.K., and Desai, B.B. (1984). *Post-Harvest Biotechnology of Fruits* (Boca Raton, Florida: CRC Press Inc.).
- Sánchez-González, L., Pastor, C., Vargas, M., Chiralt, A., González-Martínez, C., and Chafer, M. (2011). Effect of hydroxypropyl methylcellulose and chitosan coatings with and without bergamote essential oil on quality and safety of cold-stored grapes. *Postharvest Biol. Technol.* *60*, 57–63. <https://doi.org/10.1016/j.postharvbio.2010.11.004>.
- Shao, X.F., Tu, K., Tu, S., and Tu, J. (2012). A combination of heat treatment and chitosan coating delays ripening and reduces decay in 'Gala' apple fruit. *J. Food Qual.* *35*, 83–92. <https://doi.org/10.1111/j.1745-4557.2011.00429.x>.
- Sharma, D.K., Jain, V.K., Jain, R., and Sharma, N. (2013). Post-harvest study of okra (*Abelmoschus Esculentus* (L.) fruits and phytopathological effect of associated microflora. *Int. J. Innov. Res. Rev.* *1*, 27–34.
- Soliva-Fortuny, R.C., Elez-Martínez, P., and Martín-Belloso, O. (2004). Microbiological and biochemical stability of fresh-cut apples preserved by modified atmosphere packaging. *Innov. Food Sci. Emerg. Technol.* *5*, 215–224. <https://doi.org/10.1016/j.ifset.2003.11.004>.
- Synowiec, A., Gniewosz, M., Kraśniewska, K., Przybył, J.L., Bączek, K., and Węglarz, Z. (2014). Antimicrobial and antioxidant properties of pullulan film containing sweet basil extract and an evaluation of coating effectiveness in the prolongation of the shelf life of apples stored in refrigeration conditions. *Innov. Food Sci. Emerg. Technol.* *23*, 171–181. <https://doi.org/10.1016/j.ifset.2014.03.006>.
- Veldhuizen, E.J., Tjeerdsma-van Bokhoven, J.L., Zweijter, C., Burt, S.A., and Haagsman, H.P. (2006). Structural requirements for the antimicrobial activity of carvacrol. *J. Agric. Food Chem.* *54*, 1874–1879. <https://doi.org/10.1021/jf052564y>.
- Velickova, E., Winkelhausen, E., Kuzmanova, S., Alves, V.D., and Moldão-Martins, M. (2013). Impact of chitosan-beeswax edible coatings on the quality of fresh strawberries (*Fragaria ananassa* cv. Camarosa) under commercial storage conditions. *LWT-Food Sci. Technol.* *52*, 80–92. <https://doi.org/10.1016/j.lwt.2013.02.004>.
- Xiao, C., Zhu, L., Luo, W., Song, X., and Deng, Y. (2010). Combined action of pure oxygen pretreatment and chitosan coating incorporated with rosemary extracts on the quality of fresh-cut pears. *Food Chem.* *121*, 1003–1009. <https://doi.org/10.1016/j.foodchem.2010.01.038>.

Received: Jun. 20, 2017

Accepted: Oct. 25, 2017