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ORIGINAL ARTICLE

Agar-agar a promising edible coating agent for management of postharvest diseases and improving banana fruit quality

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Abstract

This study was executed to investigate the potential of agar-agar, a nontoxic and non-degradable gelling agent, as a promising coating agent to improve and protect banana fruit against fungal postharvest diseases i.e., crown, finger, neck and flower end rots which are caused by fungal isolates of *Colletotrichum musae* and *Fusarium moniliforme*. Coated-banana fruit samples with different concentrations of agar-agar suspension particularly at 2.0 g · l^{-1} exhibited a significant reduction in incidence and severity of postharvest diseases compared to untreated fruit. Banana fruits dipped in agar suspension at 2.0 g · l^{-1} for 5, 10 and 15 min showed significant reduction in disease incidence and severity. Moreover, application of agar suspension as a coating agent at 2.0 g · l^{-1} significantly decreased weight loss (%), firmness loss (%), and soluble solid concentration of banana fruit for 15 days at 25 ± 2°C. Scanning electron microscopy observation confirmed that the fruit coated with agar colloid at 2.0 g · l^{-1} had significantly fewer cracks and showed smoother surfaces than untreated fruit. This explains the quality improvement in agar-coated fruit compared to uncoated fruit. Overall, agar colloid, a safe coating agent, could be used to protect banana fruit against postharvest rot diseases and extend fruit storage life during ripening and storage.

Keywords: agar, banana, disease, fruit, fungi, storage

Introduction

Banana (*Musa* spp.) is one of the most popular fruits worldwide. Ripening banana fruit has a relatively short postharvest shelf-life due to fungal infection (Hossain and Iqbal 2016). *Colletotrichum musae* and *Fusarium moniliforme* are the major fungi causing postharvest diseases of banana fruit i.e., crown rot, finger rot, neck rot and flower end rot (Zoeir *et al.* 2017a). Application of fungicides is expensive, has harmful effects on human health and the environment, and becomes less effective after prolonged use (Lassois *et al.* 2008). Fungicide alternatives such as essential oils, salts, organic acids and chitosan have been used for controlling postharvest rot diseases of banana fruit (Hossain and Iqbal 2016; Zoeir *et al.* 2017b). Natural coating agents are composed of polysaccharides, proteins, lipids, and composites. The most common edible coating agents include waxes, chitosan, gelatin and gums which help suppress decay during postharvest storage and improve fruit quality (Ali *et al.* 2010; Cháfer *et al.* 2012; Dhall 2013). Agar is a food additive of universal use considered in the USA as GRAS (Generally Recognized as Safe) by the FDA (Food and Drug Administration) (FDA 1972). Agaro-polysaccharides can be used as a functional food to prevent inflammatory diseases (Enoki *et al.* 2010). Moreover, enzymatic hydrolysis is not relevant since there are few agarases (enzymes

that break down agaroses), found only in marine bacteria, in a few bacilli that are not normally found in food products (Armisen and Galatas 2000). Since it is non-degradable by microorganisms it is used in the preparation of microbiological and plant tissue culture media. The aim of this study was to evaluate agar-agar suspension as an edible coating agent for the management of postharvest rot diseases of banana fruit while maintaining fruit quality.

Materials and Methods

Plant material and pathogenic fungi

Healthy ripe uniform banana fruits (*Musa acuminata* cv. Balady), the most susceptible cultivar to postharvest rot diseases were obtained from orchards in El-Gharbiya Governorate, Egypt. Highly aggressive isolates of *Colletotrichum musae* and *Fusarium moniliforme*, which cause major postharvest diseases of banana fruit (Zoeir *et al.* 2017a), were provided by the Agricultural Botany Department, Faculty of Agriculture, Tanta University, Tanta, Egypt.

Source and preparation of agar suspensions

Agar-agar, a polymer made up of a subunit of galactose sugar extracted from red-purple marine algae, mainly *Gelidium amansii*, was provided by the Chemical Industrial Development Company (CID), Egypt. Different concentrations of agar colloid at 0.5, 1.0 and 2 $g \cdot l^{-1}$ (w/v) were prepared by dissolution of each amount of agar in boiling distilled water for 1–2 min, then cooled to 50°C.

Effect of agar colloid on postharvest diseases of banana fruits

Banana fruits cv. Balady were disinfected by double immersion in 2% of 70% ethanol for 5 min and allowed to dry at room temperature under sterile conditions. Fruit samples were placed separately in polyethylene bags that had been previously disinfected with 70% ethanol and exposed to UV light for 20 min. Fruit samples were dipped in different concentrations of agar colloids, 0.0, 0.5, 1.0 and 2.0 $g \cdot l^{-1}$ for 5 min at 40–50°C. In addition, different dipping times (5, 10 and 15 min) of banana fruit in agar suspension (2 $g \cdot l^{-1}$) at 40–50°C were tested. Banana fruit samples were infested by mixtures (1 : 1) of $10^6 \cdot ml^{-1}$ spore suspensions of either *C. musae* or *F. moniliforme*. Ten fruits were used as replicates and ten fruit free treatments served as a control. Fruit samples were incubated at 23 ± 2°C for 15 days. Postharvest disease incidence was calculated of diseased fruits showing symptoms of crown rot, neck rot, finger rot and flower end rot according to Zoeir *et al.* (2017a, b) as follows:

$$\text{Disease incidence } [\%] = \frac{\text{Number of diseased fruits}}{\text{Total number of banana fruit}} \times 100.$$

Disease severity was ranked by observing the percentage of rotten symptoms based on a linear scale from (0–4) as follows:

0 = healthy fruit, free of rot and discoloration,
1 = 1–25% rotten and discoloration area,
2 = 26–50% rotten and discoloration area,
3 = 51–75% rotten and discoloration area,
4 = 76–100% rotten and discoloration area.

Determination of some physicochemical properties of banana fruits

For weight loss determination, five fruit samples in each replicate for each treatment were marked before storage and weighed using a digital balance. The same fruit samples were weighed at the beginning of the experiment and after storage period s of 0, 10 and 15 days. The fruit weight loss percentage was calculated using the following formula (El-Sharony and Amin 2015):

$$\text{Weight loss } [\%] = \frac{W_1 - W_2}{W_1} \times 100,$$

where: W_1 and W_2 – initial weights and weight at specific intervals (5, 10 and 15 days), respectively.

Peel tissues from one side of banana fingers were removed and pulp firmness measurements were taken at three different points using a fruit firmness tester (Fruit Pressure Tester). Firmness values were the force required (kg) for complete penetration of 1 cm (Ranasinghe *et al.* 2005). Firmness loss (%) was calculated as follows:

$$\text{Firmness loss } [\%] = \frac{F_1 - F_2}{F_1} \times 100,$$

where: F_1 and F_2 – initial firmness and firmness at specific intervals (5, 10 and 15 days), respectively.

Soluble solid concentration (SSC) content of banana fruit pulp was estimated using Abbe's refractometer. According to Akter *et al.* (2013), a drop of banana juice squeezed from the fruit pulp on the prism of the refractometer and the percent of soluble solid content were recorded as % Brix from direct reading of the instrument. Temperature corrections were made using the temperature correction chart that accompanied the instrument.

Scanning electron microscopy (SEM) observations

Discs (4 mm) of the cortex of banana fruit that had been previously dipped in agar suspension (2 g · l^{-1} for 10 min) and artificially infested with the causal organisms, then incubated at 23–25°C for 5 days were scanned by Scanning Electron Microscopy quanta FEG250 field emission at the National Research Center unit, Egypt as follows: cortex discs were fixed in buffered osmium tetroxide (2%), then dehydrated by a graded series of ethanol (25, 50, 75, and two 100%) once for 10 min at each step, then coated with gold and viewed by SEM (Pathan *et al*. 2010).

Statistical analysis

The experiment was conducted using a completely randomized design with three replications. Data set was statistically analyzed by analysis of variance (ANOVA) technique using computer software SAS program (SAS Institute, Cary, NC, USA). Duncan's multiple range tests were used to compare differences between treatments at $p \leq 0.05$ of each variable (Snedecor and Cochran 1980).

Results

Effect of agar concentrations on banana postharvest diseases

Three concentrations of agar suspension i.e., 0.5, 1.0 and 2.0 g · l^{-1} were tested against postharvest disease incidence (%) by dipping banana fruit cv. Balady for 5 min before artificial infestation by causal pathogen inocula of *C. musae* and *F. moniliforme*. Data (Fig. 1) indicated that all agar concentrations significantly suppressed postharvest rot diseases of banana fruit compared to control 10 days after infestation at 25 ± 2°C. In addition, all agar concentrations suppressed disease incidence (%) and severity of crown, neck, finger and flower end rot compared to control 15 days after infestation (Table 1). It was observed that the reduction of postharvest disease incidence and severity was increased with increasing agar concentrations. An agar suspension at 2 g · l^{-1} was the best treatment for reducing incidence and severity of all postharvest diseases of banana fruit.

Control 0.5 g · l^{-1} 1.0 g · l^{-1} 2.0 g · l^{-1}

Fig. 1. Effect of different concentrations of agar suspensions on postharvest diseases of banana fruit after 10 days of storage at 25 ± 2°C

Table 1. Effect of different agar suspension concentrations on postharvest disease incidence (%) and severity (DS) of banana fruit 15 days after infestation at 25 ± 2°C

Treatment	Postharvest rot disease incidence [%] and severity (DS)							
	crown rot		neck rot		finger rot		flower end rot	
	[%]	DS	[%]	DS	[%]	DS	[%]	DS
Control	100.0 a	4.0 a	100.0 a	4.0 a	100.0 a	4.0 a	100.0 a	4.0 a
Agar [0.5 g · l^{-1}]	100.0 a	4.0 a	60.0 b	3.0 b	100.0 a	4.0 a	70.0 c	3.0 b
Agar [1.0 g · l^{-1}]	80.0 b	4.0 a	60.0 b	3.0 b	100.0 a	4.0 a	60.0 b	3.0 b
Agar [2.0 g · l^{-1}]	40.0 c	2.0 b	20.0 c	1.0 c	30.0 b	2.0 b	20.0 d	1.0 c

Values in each column followed by the same letter are not significantly different at $p \leq 0.05$ according to Duncan's multiple range

Effect of dipping time of banana fruit in agar suspension on banana postharvest diseases

Different dipping times of banana fruit i.e., 5, 10 and 15 min in agar suspension at 2 g · l^{-1} were tested (Table 2). Data obtained indicated that different dipping times of banana fruit in agar colloid (2 g · l^{-1}) significantly reduced all postharvest rot diseases of banana fruit compared to control 15 days after artificial infestation by causal pathogens. Moreover, the disease incidence (%) and severity decreased with increasing dipping times (Table 2).

Table 2. Effect of different dipping times in agar suspension (2 g · l^{-1}) on postharvest rot disease incidence and severity of banana fruit 15 days after infestation at 25 ± 2°C

Treatment	Dipping time [min]	Postharvest rot disease incidence [%] and severity (DS)							
		crown rot		neck rot		finger rot		flower end rot	
		[%]	DS	[%]	DS	[%]	DS	[%]	DS
Control	–	100 a	4.0 a	70.0 a	3.0 a	100 a	4.0 a	80.0 a	4.0 a
Agar colloid	5	70.0 b	3.0 b	50.0 b	2.0 b	80.0 b	4.0 a	30.0 b	2.0 b
[2 g · l^{-1}]	10	50.0 c	2.0 c	40.0 c	2.0 b	0.0 c	0.0 b	0.0 c	0.0 c
	15	50.0 c	2.0 c	30.0 d	2.0 b	0.0 c	0.0 b	0.0 c	0.0 c

Values in each column followed by the same letter are not significantly different at p ≤ 0.05 according to Duncan's multiple range

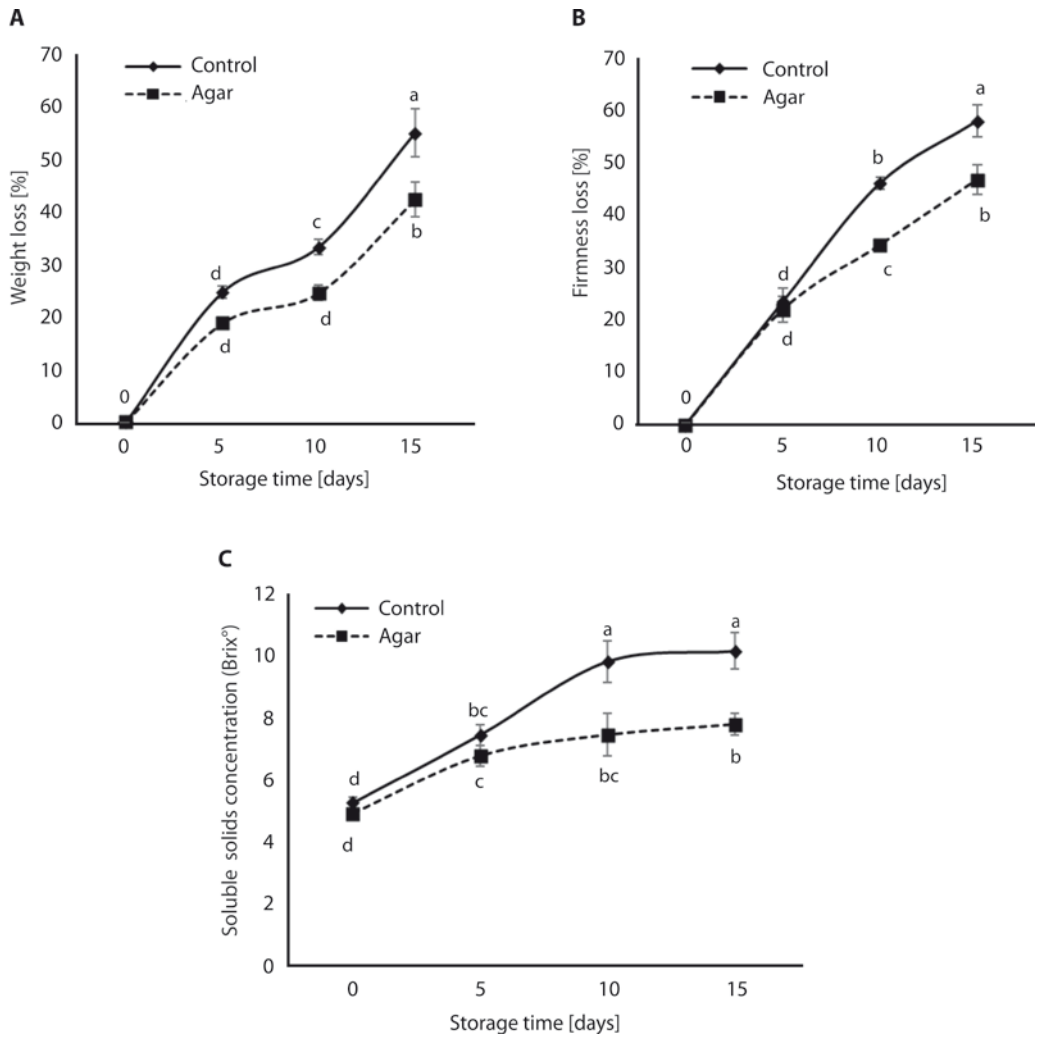

Fig. 2. Effect of agar suspension [2 g · l^{-1}] on (A) weight loss [%], (B) firmness loss [%] and (C) soluble solid concentration (Brix°) of banana fruit during different storage time (5, 10 and 15 days) at 25 ± 2°C (Values are means ± SE. Values followed by the same letter are not significantly different at p ≤ 0.05 according to Duncan's multiple range)

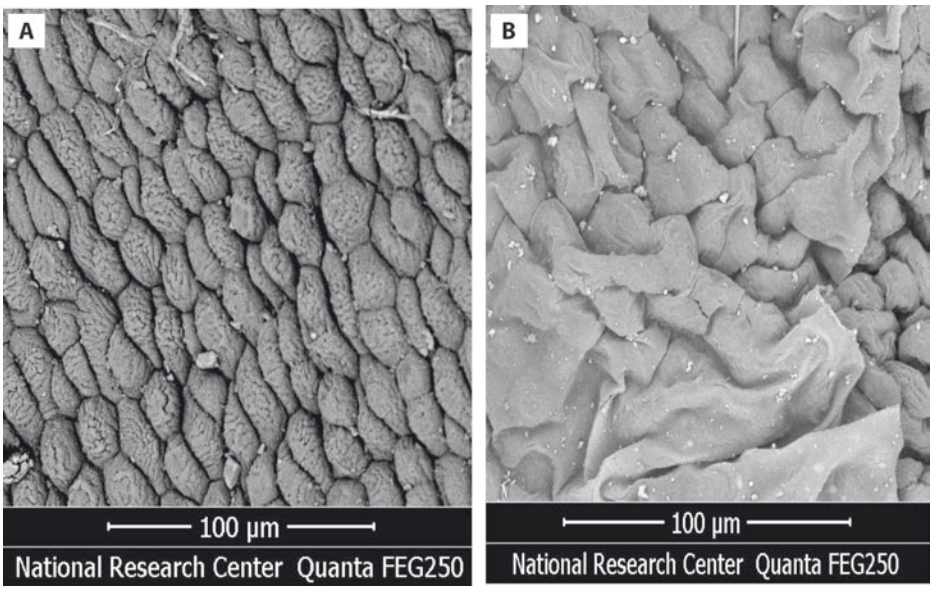

Fig. 3. Scanning Electron Microscopy (SEM) observation on the surface of (A) uncoated banana fruit and (B) coated banana fruit with agar suspension at 2 g · l^{-1} by dipping for 10 min

Effect of agar coating on physiochemical properties of banana fruits

Weight loss (%), firmness loss (%) and soluble solid concentration (SSC) of coated and uncoated banana fruit gradually increased with increasing storage periods (Figs. 2A–C). Banana fruit coated with agar colloid at 2 g · l^{-1} for 10 min had less weight loss and firmness loss percentages and SSC than control (uncoated) fruit during storage periods (10 and 15 days). At the end of storage, agar-coated fruit clearly showed the lowest weight loss and firmness loss percentages and SSC compared to control fruit (Figs. 2A–C).

Scanning electron microscopy (SEM) observations

Banana fruit coated with agar colloid at 2 g · l^{-1} for 10 min at 40–50°C, which had been artificially inoculated with pathogenic fungi as well as control fruit, were investigated by scanning electron microscopy (SEM) as shown in Figure 3. Results indicated that the surface of banana fruit had significantly fewer cracks and smoother surfaces as well as limited mycelia of pathogenic fungi compared with the untreated fruit. Thus, agar could be used as a protective agent against postharvest diseases.

Discussion

Increasing awareness of health problems and environmental issues due to application of pesticides has led to studies on safe, eco-friendly and cost-effective fruit preservation techniques to maintain quality and extend the shelf-life of fruit (Maqbool *et al.* 2010). Over the last two decades, the development and use of edible coatings to prolong the shelf-life and improve fruit quality has been intensively researched (Dhall 2013). Agar-agar, a hydrophilic colloid extracted from marine red algae (Davidson 2006), has multiple applications particularly in the human food industry. Since it is non-degradable by microorganisms, agar-agar is commonly used as a solidifying agent in the preparation of microbiological and plant tissue culture media (Armisen and Galatas 2000). The present study demonstrated that agar suspensions at different concentrations, especially at 2 g · l^{-1}, applied by dipping banana fruit for 10 min could be a promising protective coating agent for management of postharvest rot diseases. The lowest concentrations of agar suspension (0.5 and 1 g · l^{-1}) had less efficacy than 2 g · l^{-1}. This could be because low concentrations of agar coating were less thick and did not completely cover fruit surfaces. The same results were obtained by Ali *et al.* (2010) for tomato fruit coated with gum Arabic. Agar as a polysaccharide coating agent resembles chitosan and gum Arabic for controlling postharvest diseases. Chitosan was effective in controlling postharvest diseases in litchi (Zhang and Quantick 1997), sweet cherries (Romanazzi *et al.* 2012) and banana fruits (Hossain and Iqbal 2016). Fruit weight loss during storage is attributed to increased water loss (transpiration) and respiration (Yaman and Bayoindirli 2002). The weight loss reduction of agar-coated banana fruit compared to

control fruit was probably due to the effect of agar as a barrier against gas exchange (O_2 and CO_2) and water vaporization (Baldwin *et al.* 1999; Park 1999). It thus reduces transpiration and respiration and thereby reduces fruit weight loss compared to control fruit. Accordingly, the extended storage life of banana fruit coated with agar colloid could be due to the modification of the internal atmosphere and water loss reduction (Ali *et al.* 2010, 2011). The present study revealed that agar application significantly reduced firmness loss of banana fruit compared to control (Yaman and Bayoindirli 2002). Limited respiration in coated fruit decreases the activity of cell wall hydrolases, and thereby, delays fruit ripening and reduces fruit firmness loss (Tanada-Palmu and Grosso 2005). Soluble solid concentration (SSC) was increased with increasing storage periods due to ripening progress that is attributed to ethylene production. However, SSC was lower in coated fruit than control particularly at the end of the storage period. As mentioned earlier, coated fruit has a limited respiration rate compared to control fruit. In addition, the decreased respiration rate slows down ethylene production resulting in lower SSC (Yaman and Bayoindirli 2002). The effect of agar suspensions as a coating agent to protect banana fruit against postharvest rot fungi while maintaining fruit quality was explained by scanning electron microscopy (SEM) observations. The results indicated that agar is a good film covering of banana fruit that limited mycelial growth and lessened cracking of fruit surfaces. This could explain the effect of agar suspensions of reducing fruit weight loss, firmness loss and fruit infections by pathogenic fungi, and in turn, the extension of the shelf-life of banana fruit.

Conclusions

The present study concluded that using an agar suspension as a coating agent could be a promising, safe and cheap approach to extend shelf-life of banana fruit through its role in enhancing fruit physico-chemical properties and reducing the incidence and severity of postharvest diseases of banana fruit during storage. Further studies should be conducted to investigate the effect of agar suspension, as a coating agent, on physiological processes of fruit ripening, in addition to its combination with other safe fungicide alternatives on other banana cultivars and crop fruits.

References

Akter H., Hassan M.K., Rabbani M.G., Mahmud A.A. 2013. Effects of variety and postharvest treatments on shelf life and quality of banana. Journal of Environmental Science and Natural Resources 6 (2): 163–175. DOI: http://dx.doi.org/10.3329/jesnr.v6i2.22113

Ali A., Maqbool M., Ramachandran S., Alderson P.G. 2010. Gum arabic as a novel edible coating for enhancing shelf-life and improving postharvest quality of tomato (*Solanum lycopersicum* L.) fruit. Postharvest Biology and Technology 58 (1): 42–47. DOI: https://doi.org/10.1016/j.postharvbio.2010.05.005

Ali A., Mahmud T.M.M., Sijam K., Siddiqui Y. 2011. Effect of chitosan coatings on the physico-chemical characteristics of Eksotika II papaya (*Carica papaya* L.) fruit during cold storage. Food Chemistry 124 (2): 620–626. DOI: https://doi.org/10.1016/j.foodchem.2010.06.085

Armisen R., Galatas F. 2000. Agar. p. 21–40. In: "Handbook of Hydrocolloids" (G.O. Phillips, P.A. Williams, eds.). CRC Press., Cambridge, England, 450 pp.

Baldwin E.A., Burns J.K., Kazokas W., Brecht J.K., Hagenmaier R.D., Bender R.J., Pesis E. 1999. Effect of two edible coatings with different permeability characteristics on mango (*Mangifera indica* L.) ripening during storage. Postharvest Biology and Technology 17 (3): 215–226. DOI: https://doi.org/10.1016/S0925-5214(99)00053-8

Cháfer M., Sánchez-González L., González-Martínez C. 2012. Fungal decay and shelf life of oranges coated with chitosan and bergamot, thyme, and tea tree essential oils. Journal of Food Science 77 (8): 182–187. DOI: 10.1111/j.1750-3841.2012.02827.x.

Davidson A. 2006. The Oxford Companion to Food. Oxford University Press. 936 pp. DOI: 10.1093/acref/97801.92806819.001.0001.

Dhall R.K. 2013. Advances in edible coatings for fresh fruits and vegetables: A review. Critical Reviews in Food Science and Nutrition 53 (5): 435–450. DOI: 10.1080/10408398.2010.541568

Enoki T., Okuda S., Kudo Y., Takashima F., Sagawa H., Kato I. 2010. Oligosaccharides from agar inhibit pro-inflammatory mediator release by inducing heme oxygenase 1. Bioscience, Biotechnology, and Biochemistry 74 (4): 766–770. DOI: 10.1271/bbb.90803

El-Sharony T.F., Amin O.A. 2015. Effect of some natural substances on fruit quality of Washington navel orange under cold storage. Journal of Agriculture and Ecology Research International 2 (1): 58–68. DOI: 10.9734/JAERI/2015/12222

FDA. 1972. Agar-agar, GRAS (generally recognized as safe) Food Ingredients. Food and Drug Administration, PB-265502, Federation of America Societies for Experimental Biology, Bethesda MD, USA.

Hossain M.S., Iqbal A. 2016. Effect of shrimp chitosan coating on postharvest quality of banana (*Musa sapientum* L.) fruits. International Food Research Journal 23 (1): 277–283.

Lassois L., de Bellaire L., Janaki M.H. 2008. Biological control of crown rot of bananas with *Pichia anomala* strain K and *Candida oleophila* strain. Biological Control 45 (3): 410–418. DOI: https://doi.org/10.1016/j.biocontrol.2008.01.013

Maqbool M., Ali A., Ramachandran S., Smith D.R., Alderson P.G. 2010. Control of postharvest anthracnose of banana using a new edible composite coating. Crop Protection 29 (10): 1136–1141. DOI: https://doi.org/10.1016/j.cropro.2010.06.005

Park H.J. 1999. Development of advanced edible coatings for fruits. Trends in Food Science and Technology 10 (8): 254–260. DOI: https://doi.org/10.1016/S0924-2244(00)00003-0

Pathan A.K., Bond J., Gaskin R.E. 2010. Sample preparation for SEM of plant surfaces. Materials Today 12 (1): 32–43. DOI: https://doi.org/10.1016/S1369-7021(10)70143-7

Ranasinghe L.S., Jayawardena B., Abeywickrama K. 2005. An integrated strategy to control post-harvest decay of Embul banana by combining essential oils with modified atmosphere packaging. International Journal of Food Science and Technology 40 (1): 97–103. DOI: https://doi.org/10.1111/j.1365-2621.2004.00913.x

Romanazzi G., Lichter A., Gabler F.M., Smilanick J.L. 2012. Recent advances on the use of natural safe alternatives to conventional methods to control postharvest gray mold of table grapes. Postharvest Biology and Technology 63 (1): 141–147. DOI: https://doi.org/10.1016/j.postharvbio.2011.06.013

Snedecor G.W., Cochran W.G. 1980. Statistical Methods. 7th ed. Iowa State Univ. Press, Ames, 503 pp.

Tanada-Palmu P.S., Grosso C.R.F. 2005. Effect of edible wheat gluten-based films and coatings on refrigerated strawberry (*Fragaria ananassa*) quality. Postharvest Biology and Technology 36 (2): 199–208. DOI: https://doi.org/10.1016/j.postharvbio.2004.12.003

Yaman O., Bayoindirli L. 2002. Effects of an edible coating and cold storage on shelf-life and quality of cherries. LWT – Food Science and Technology 35 (2): 146–150. DOI: https://doi.org/10.1006/fstl.2001.0827

Zhang D., Quantick P.C. 1997. Effects of chitosan coating on enzymatic browning and decay during postharvest storage of litchi (*Litchi chinensis* Sonn.) fruit. Postharvest Biology and Technology 12 (2): 195–202. DOI: https://doi.org/10.1016/S0925-5214(97)00057-4

Zoeir H.A., El Zahaby H.M., Ziedan E.H., Maswada H.F. 2017a. Etiology and ecology of fungi causing postharvest diseases of banana fruits in Egypt. Plant Archives 17 (2): 1463–1468.

Zoeir H.A., El Zahaby H.M., Ziedan E.H., Maswada H.F. 2017b. Efficacy of antifungal activity of essential oils, salts and antioxidants acids on pathogenic fungi and their application methods for controlling postharvest diseases on banana fruits. African Journal of Microbiology Research 11 (29): 1162–1170. DOI: 10.5897/AJMR2017.8607