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RESEARCH REPORT

Postharvest Technology

Prolonging the shelf life of 'Agege Sweet' orange with chitosan–rhamnolipid coating

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Abstract
This study evaluates the single and combined usage of chitosan (2% w/v) and rhamnolipid (2% w/v) as edible coatings to extend the shelf life of sweet oranges stored at 25 °C for 8 weeks. Physiochemical, microbial and sensory analysis of the oranges was conducted during ambient storage. The combined treatment of chitosan and rhamnolipid coating on oranges significantly delayed a loss in chlorophyll quality, malondialdehyde, weight loss, soluble solids content, titratable acidity, vitamin C content and delayed the loss of firmness during the 8 weeks of storage. The combined chitosan–rhamnolipid coating significantly increased the activities of superoxide dismutase, catalase, and peroxidase, as well as inhibited the generation of superoxide free radicals and the growth of mesophilic bacteria, yeast and mould.

Keywords *Citrus sinensis* · Edible coating · Rhamnolipid · Chitosan film · Microbial count

1 Introduction

Fruits are excellent dietary sources of nutrients, micronutrients, vitamins, fibre and minerals that are important for normal function and maintenance of human health. These fruit components are important because of their ability to prevent vitamin C and A deficiencies, as well as reduce the risk of several diseases (Bello et al. 2016). However, a major challenge facing the orange industry in particular is rot diseases caused by fungal pathogens, which contribute to severe yearly losses of agricultural and horticultural crops because they minimize orange fruit shelf life, and thereby reduce their economic value (Parveen et al. 2016). The global orange production for years 2015/2016 was forecasted to be 3.0 million metric tons, which was down from the 45.8 million tons reported for the previous year in Brazil, the United States, and Mexico (FAS/USDA 2016).

Edible coatings are one of the techniques currently used for the postharvest management of fruits because it increases the shelf-ife of fruit. Edible coatings prevent microbial spoilage and prevent their adverse effect on fruits during storage (Correa-Betanzo et al. 2011; Moreira et al. 2011). Coatings form a layer of biological polymers on fruits, which consequently caused a reduction in their respiration rates and water loss during storage (Adetunji et al. 2013; Hamzah et al. 2013). Also, their biodegradability and eco-friendly nature gave a good priority over the conventional synthetic polymers (Del-Valle et al. 2005). Bioactive ingredients can be incorporated into edible films so as to prolong shelf life of processed or whole fruit (Sipahi et al. 2013).

Many biopolymers have been used as edible coatings, including chitosan (Rojas-Grau et al. 2009), carboxymethyl cellulose (Arnon et al. 2014) and chitosan (Adetunji et al. 2014). Rhamnolipids are microbial biosurfactants with many

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useful applications in pharmaceutical, food, cosmetics and detergent industries, and also agriculture, where they are mostly explored for plant pathogen elimination. The major reason behind the wide acceptability of rhamnolipids is due to their non-toxicity, surfactant features, their antimicrobial properties against many microbes, and their "eco-friendly" properties (Sekhon et al. 2014; Silva et al. 2015).

Different researchers have validated the use of different preservative techniques including chemical fungicides, parking houses and refrigeration for the postharvest management of citrus fruits. Most farmers, consumers, handlers and processors cannot afford the cost to maintain controlled atmospheric storage facilities (FAO 2017). These challenges are coupled with costly and irregular supplies of power or electricity in most developing countries (Dillon and Barrett 2014). Furthermore, most of the orchards are located in rural areas without any electricity, which adds to the problem of using postharvest storage devices and normally leads to post-harvest losses in most developing countries (Sheahan and Barrett 2017).

The continual application of synthetic pesticides has led to various ecological imbalance in the management of decay and pests during the pre- and post-harvest of fruits and vegetables (Shrivastava et al. 2017). The application of these chemically synthesized compounds to fruits and vegetables has led to and increased bioaccumulation and biomagnification of the chemicals with subsequent health hazards and environmental pollution, thereby increasing greenhouse effects (Adetunji et al. 2018). Moreover, due to increased public concern on food safety, there is a need for a natural, safe and environmentally friendly means of controlling pests and diseases, most especially from biological origins (Meng et al. 2010). The novelty of the work is based on the fact that rhamnolipids have not been used before in combination with chitosan to extend the shelf life of fruits, although the role of both products is well known as post-harvest technologies (Chien et al. 2007a; Nehal et al. 2012; Mohammed et al. 2016). The present study was intended to assess the effect of 2% chitosan alone or in combination with 2% rhamnolipids, to improve the post-harvest management of orange fruit.

2 Materials and methods

2.1 Plant material

Fully matured, green coloured and thick wax covered local cultivar of sweet oranges 'Agege sweet' was harvested from a commercial orchard in the Ogbomosho town (8°8′0″N, 4°16′0″E) in the Oyo State of Nigeria and immediately transported to the postharvest laboratory at the Nigerian Stored Product Research Institute (NSPRI) in Ilorin (8°30′0″N, 4°33′0″E), Kwara State, Nigeria. During the selection of oranges for the experiment, factors such as uniformity in size, colour, shape, greenness, and absence of injury were considered, and blemished and diseased fruits were removed. The orange fruits were disinfected with a chlorine solution (0.0003%) for 30 s, washed under running tap water, and then cleaned with sterilized water.

2.2 Preparation of chitosan

Matured brownish crabs were collected from the brackish waters of Warri in the Delta State of Nigeria. The animals were sacrificed and the viscera and muscles were carefully removed. The shells were dehydrated at 60 °C overnight and milled with a grinder that was fabricated locally at Postharvest Engineering Research Laboratory, NSPRI in Ilorin. The methods established by Pandharipande and Prakash (2016) were used to obtain chitin from crab shell waste and the method described by Adetunji et al. (2014) was used for further treatment of chitin to produce the chitosan used for this study. Subsequent washing and drying steps yielded chitosan.

2.3 Microorganisms and growth conditions

The bacteria strain C1501 was isolated from the rhizosphere of a wheat plants grown at the research farm of NSPRI, Ilorin Kwara States. The isolated strain was identified as *Pseudomonas aeruginosa* using 16 s rRNA gene sequencing and coded C1501 with the accession number KF976394. 16S rDNA gene sequencing was submitted to Gene Bank (http;//www.ncbi.nlm.nih.gov). Strain C1501 was incubated at 37 °C for 48 h on King's agar in a Biological Oxygen Demand Incubator and later stored in at 4 °C (Adetunji et al. 2017a).

2.4 Preparation of culture and fermentation media

The rhamnolipid producing strain was cultured on Cetrimide agar plates at 37 °C for 14–16 h. Approximately 50 mL of the liquid medium was then used for inoculum development of strain C1501. This solution was then cultured at 37 °C with shaking at 200 rpm for 14–16 h. The method described by Onkar et al. (2015) was subsequently followed to prepare the liquid fermentation medium. The submerged fermentation was performed with 40 $g{\cdot}L^{-1}$ glucose as a carbon source in the basal medium described by Rufino et al. (2014). The fermentation was carried out at 37 °C with shaking at 200 rpm, with pH of 6.8 following the procedure described by Moussa et al. (2014).

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2.5 Extraction of rhamnolipids

The pH of the remaining supernatant from the fermentation medium was adjusted to 2.0 with 1 N hydrochloric acid to precipitate rhamnolipids. The resulting precipitation was recovered by centrifugation at 9000×*g* for 20 min, and then extracted three times with ethyl acetate (2:1% v/v). The organic phase was separated and concentrated in a rotary evaporator, which later formed the rhamnolipids (Saravanan and Subramaniyan 2014). The crude rhamnolipids product was then purified using the chromatographic procedures developed by Darvishi et al. (2011). The solvent used to elute the crude rhamnolipids was n-hexane in a column (1.5 cm × 35 cm) made of silica gel 60. The following solvents, hexane, acetone, chloroform, chloroform, methanol were later used for elution of the rhamnolipid in increasing order of polarity (Darvishi et al. 2011). Thin layer chromatography was used for the separation of the various fractions of the rhamnolipid after concentrating them in a rotary evaporator in a solvent system containing chloroform: methanol: 20% aqueous acetic acid in ratio of 65:15:2 (Franzetti et al. 2014). The resultant rhamnolipids extract was confirmed with the aid of specific reagents for sugars and fatty acids on thin layer chromatography (Darvishi et al. 2011; Wittgens et al. 2011).

2.6 Purification and characterization of rhamnolipid

The obtained crude extract was dissolved in 50% ethanol, after which it was filtered through an ultrafiltration membrane with a 30-kDa molecular weight cut-off. The filtrate obtained after ultrafiltration was precipitated with acid, dissolved in sterilized deionized water and filtered through a 1-kDa membrane. This procedure was repeated to obtain the final precipitate that was lyophilized in order to obtain purified rhamnolipid. This product was dissolved in deutrimethanol and the purified rhamnolipid was characterized using liquid chromatography/mass spectrometry and nuclear magnetic resonance spectroscopy techniques established by Adetunji et al. (2017b).

2.7 Treatments and storage conditions of the oranges

The orange fruits were coated with four of the following treatments: oranges in the first (T1) and the second groups (T2) were coated with 2% chitosan and 2% rhamnolipid alone, respectively. Fruits in the third group (T3) were coated with the mixture of the two compounds (2% chitosan + 2% rhamnolipid (1:1)). The fourth group of oranges (T4) was left uncoated to serve as the control and were dipped in distilled water. The treatments were allowed to dry on the oranges at ambient temperature using the standard procedure developed by Jiang et al. (2005). Samples were collected from each set for analysis once every week for a total of 8 w of storage. Thereafter, the fruits were stored at 25 ± 1 °C with 70–80% relative humidity.

2.8 Firmness of fruits

The firmness of the oranges was determined using a penetrometer, which has a probe 8 mm in diameter that was pressed gradually onto the surface of each orange. The results obtained were expressed in kg cm^{-2}.

2.9 Weight loss of fruits

Weight loss of stored oranges was determined by weighing the fruits before and after each week during storage. The weight loss of stored oranges was later calculated using the formula: $(A - B)/A \times 100$, where A is the weight of the orange fruit before application of the coating material and before storage, and B is the weight of orange fruit after application of the coating material after storage.

2.10 Chlorophyll content

Chlorophyll content was measured using a standard procedure that was established by AOAC (1999).

2.11 Malondialdehyde content

Malondialdehyde content was determined using the protocol developed by Liu et al. (2007). Orange fruit pulp (3.0 g) was mashed uniformly in 7 mL 10% trichloroacetic acid followed by centrifugation the at 10,000×*g* for 15 min. Then, 2 mL of the supernatant was mixed with 6 mL 0.6% thiobarbituric acid, followed by heating of the mixture to 100 °C for 20 min and another centrifugation at 10,000×*g* for 10 min. The absorbance readings for the supernatant were determined at 450, 532 and 600 nm. The malondialdehyde concentration was evaluated using the formula: $6.45 \times (A532 - A600) - 0.56 \times A450$. Absorbance was taken in triplicate. The concentration of malondialdehyde was recorded as nmol g^{-1}.

2.12 Enzyme activity

2.12.1 Extraction of enzymes for assay

A 10 g aliquot from the inner pulp of the orange fruits from each group was removed and uniformly mashed in 10 ml 50 mM sodium phosphate buffer with a fruit juice grinder containing 1% polyvinylpolypyrrolidone and 1 mM EDTA. A 50 mM sodium phosphate solution (pH 7.5) was used as a buffer for the catalase (CAT) and superoxide dismutase

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(SOD) assays; while a 100 mM sodium phosphate buffer (pH 6.5) was used for the peroxidase (POD) assay. The supernatant used for the assay was obtained by centrifuging the fruit homogenate at 27,000×*g* for 50 min at 4 °C.

2.12.2 Assay for enzyme activities

Evaluation of CAT and SOD activities was carried out using the protocol developed by Wang et al. (2005). The absorbance value of the treated oranges was taken at 240 nm and the activity of SOD was expressed in U g^{-1}. The catalase activity was determined by the amount of one unit of catalase enzyme required for the conversion of 1 M hydrogen peroxide from the pulp of each orange fruit tested per minute at 30 °C. The absorbance for SOD was evaluated at 560 nm. The SOD activity was defined as the total amount of enzyme required to cause a 50% decrease of nitro blue tetrazolium from each orange pulp sample every one hour, and was described as U g^{-1}. The protocol developed by Tian et al. (2005) was used in the determination of POD activity, which was evaluated by measuring the increased absorbance at 398 nm and 460 nm. POD activity was evaluated as the increase in absorbance of the pulp of each orange fruit tested every minute at 30 °C, and recorded as U g^{-1}.

2.13 Reactive oxygen species ($O_2^{\bullet-}$)

The measurement of ROS was performed using a protocol developed by Wang and Jiao (2000). The Reactive oxygen species ($O_2^{\bullet-}$) was determined by recording the nitrite development from hydroxylamine alongside with the formation of $O_2^{\bullet-}$. A standard curve of NO_2 was used to evaluate the release of $O_2^{\bullet-}$ from the reaction that occurs between reactive oxygen species and hydroxylamine. The absorbance was determined at 530 nm and the $O_2^{\bullet-}$ production rate was expressed as nmol $g^{-1}min^{-1}$.

2.14 Measurements of SSC, TA and ascorbic acid content in pulp

The soluble solids content (SSC) in fruit pulp was measured using a hand refractometer and expressed as °Brix. Titratable acid (TA) in pulp juice was assayed using the protocol from Serrano et al. (2005) with minor modifications. In this case, 5.0 g of orange pulp juice was mixed with 50 mL sterilized water and titrated with 0.1 M NaOH, which was then expressed as a percentage of citric acid. Orange pulp tissue (1.5 g) was mixed thoroughly with 20 mL 6% metaphosphoric acid in 2 M acetic acid and the resultant mixture was centrifuged for 15 min at 12,000×*g* at 4 °C to obtain an L-ascorbic acid extract. The method described by Deepa et al. (2006) using 2,6- dichlorophenolindophenol- dye was used to evaluate the total amount of L-ascorbic acid present in each orange pulp tissue sample, and the results were reported as mg of ascorbic acid equivalent to 100 g^{-1} FW.

2.15 Total microbial counts

The total microbial count was carried out by grinding 1 g of orange pulp tissue, which was then mixed thoroughly with 10 mL water and a 1:10 dilution was further serially diluted to 10^{-7}. Total aerobic mesophilic bacteria present in the samples was determined by plating 0.1 mL of the dilutions on plate count agar plates using a sterilized L shaped glass spreader. Plates were incubated at 37 °C for 48 h. Approximately 0.1 mL of each serial dilution of the orange pulp homogenate was plated on potato dextrose agar containing 100 $\mu g \cdot g^{-1}$ streptomycin and incubated at 28 °C for 5 d to evaluate yeast and mould counts. Colony forming units (CFU) per gram of orange pulp were evaluated in triplicate and designated as log CFU x g^{-1}.

2.16 Sensory evaluation

Sensory evaluation of the orange fruits was carried out by a sensory panel at the sensory evaluating laboratory of Nigerian Stored Product Research Institute. Eight trained staff of the Nigerian Stored Product Research Institute with experience in sensory evaluation of fruit evaluated fruit quality metrics including colour, freshness, taste, aroma and texture. The protocol developed by Martinez-Romero et al. (2013) for overall acceptance of the treated and untreated oranges using a five-point scale was adapted for this study (5 = like the orange fruits, 4 = moderately like the orange fruits, 3 = neither like nor dislike the orange fruits, 2 = moderately dislike the orange fruits and 1 = totally dislike the orange fruits).

2.17 Statistical analysis

All data were obtained in triplicate and evaluated by analysis of variance using SPSS v 16.0, with significant difference ($p < 0.05$) among means separated using Duncan's multiple range test.

3 Results and discussion

3.1 Firmness

The coating application from T1, T2, and T3 groups significantly prevented ($p < 0.05$) the loss of firmness in stored oranges (Fig. 1A). Firmness is one of the major factors that determine quality and shelf life of fruits during storage. The level of the loss in firmness during storage of fruit can be linked to the breaking down of cell wall components, such

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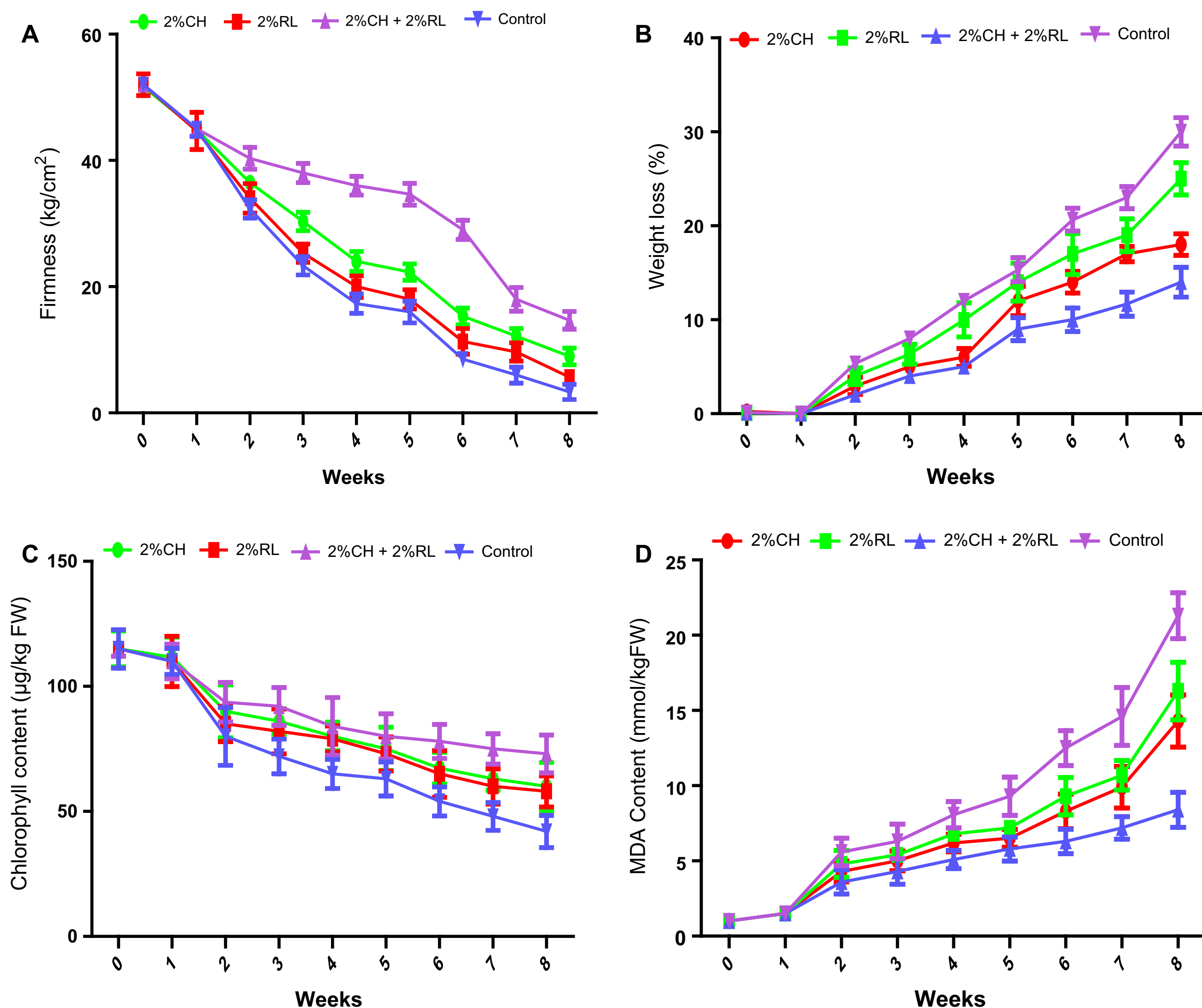

Fig. 1 Effect of chitosan and rhamnolipid coating on firmness (**a**), weight loss (**b**), chlorophyll content (**c**) and malondialdehyde content (**d**) of orange fruit during storage at 25 °C. Each value is the mean and standard errors for three replicates

as soluble pectin or pectins, due to the activity of specific pectinase enzymes such as polygalacturonase and pectin esterase that cause degradation of pectin during fruit ripening (Kashappa and Hyun 2006; Tanada-Palmu and Grosso 2005). The mode of action of these enzymes are involved in the utilization of chitosan coating in the maintenance of firmness could be linked to the antifungal activities of chitosan, which can thereby reduce infection on fruits when used as edible coatings on fruits. The coating treatment subsequently interrupts the other ripening processes during storage, and our study shows that the rate of fruit firmness loss decreases during storage when fruits are treated with chitosan and rhamnolipids coatings. These coatings prevented the loss of firmness after 5 w of the ambient storage, which was followed by fruit softening at the end of 8 w of storage. The combination of chitosan and rhamnolipids treatment (T3) was more effective in preserving the firmness of the coated oranges compared to the other treatments (Fig. 1A), which was similar to other reports of the application of chitosan coatings used as a preservative in different fruits (Ali, et al. 2011; Zhu et al. 2008).

3.2 Weight loss

Fruit weight loss was significantly lower ($p < 0.05$) in the treated fruits in groups T1, T2 and T3 when compared with the uncoated control group (Fig. 1B). The effect of the edible coating on fruit weight was more pronounced after 8 w of storage, where we observed a drastic reduction in weight loss in the treated when compared to the control fruits. Weight

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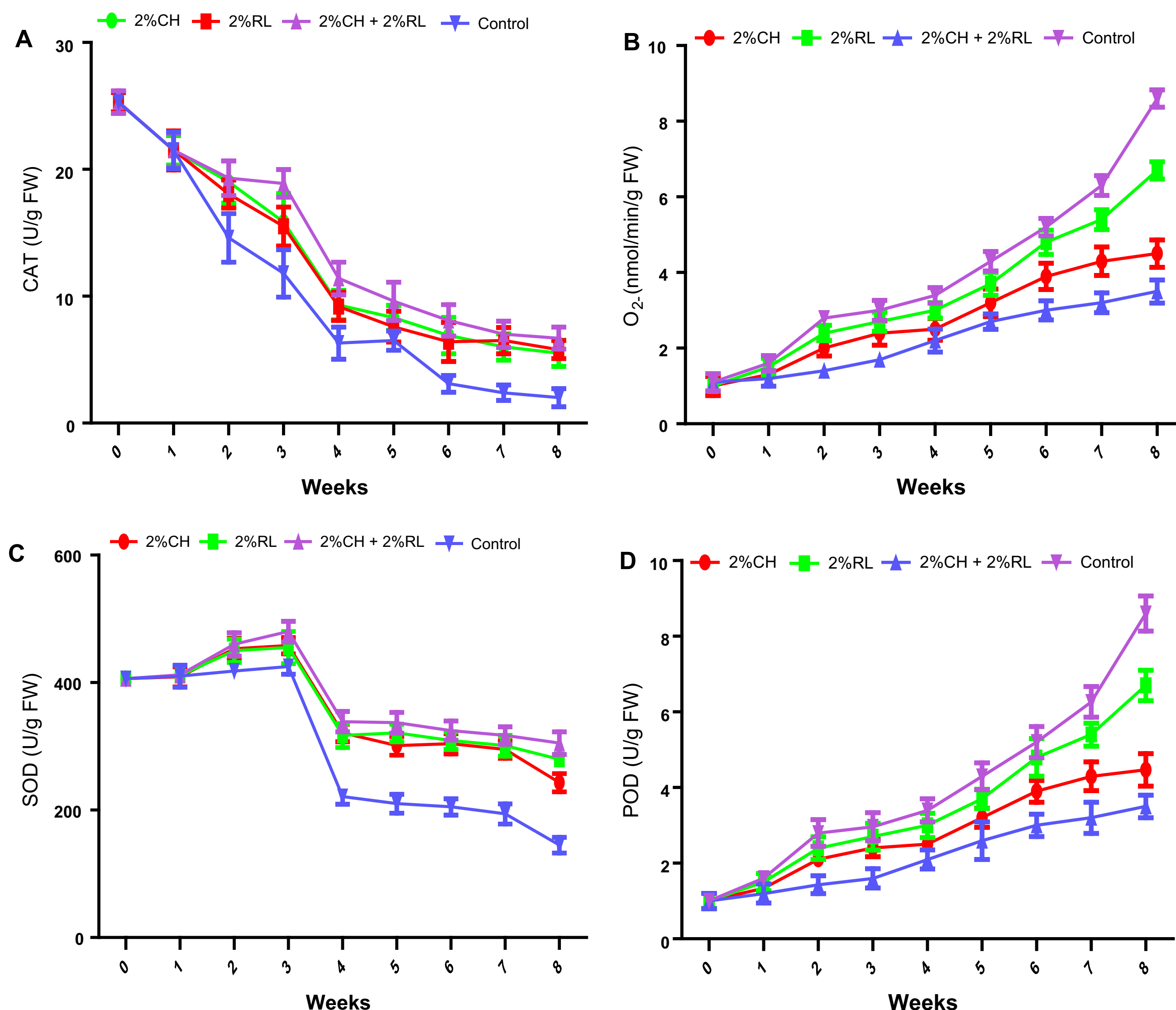

Fig. 2 Effect of chitosan and rhamnolipid coating on the activities of Catalase (**a**), Reactive *oxygen* species (**b**) Superoxide dismutase (**c**) and Peroxidase (**d**) of orange fruit during storage at 25 °C. Each value is the mean and standard errors for three replicates

loss is an important factor that determines post-harvest shelf life of fruit during storage (Liplap et al. 2013).

The T3 treatment reduced weight loss in oranges during the 8 w of storage (Fig. 1B). It was observed in this study that the treatment T3 minimized weight losses in coated oranges as compared to other treatments (Fig. 1B). This observation is similar to earlier reports of chitosan used singly or in addition to other coating materials. The significant reduction in weight loss observed whenever chitosan is applied as an edible coating during storage might be linked to its ability to form a semipermeable barrier against oxygen, carbon dioxide and moisture. This then prevents water loss, dehydration and shrinkages on the coated fruits. (Ali et al. 2011, Gol et al. 2013; Arnon et al. 2014; Valero et al. 2013).

3.3 Chlorophyll

It was observed that the colour of orange fruits changes from green to yellow during the storage period. The initial chlorophyll content of the orange fruits was 110 $\mu g \cdot g^{-1}$, and decreased with storage time. Also, there was a substantial difference ($p < 0.05$) between the chlorophyll content of the coated fruits in the T1, T2 and T3 treatment groups, as well as in uncoated samples towards the end of the storage period. The samples treated with combined coatings in group T3 retained their green colour (73 $\mu g \cdot g^{-1}$) longer, followed by T1 (60 $\mu g \cdot g^{-1}$) and T2 (58 $\mu g \cdot g^{-1}$), compared to the control group (42 $\mu g \cdot g^{-1}$), which had completely changed to yellow by the end of the storage period (Fig. 1C). It was observed in this study that the T3 treatment retarded colour

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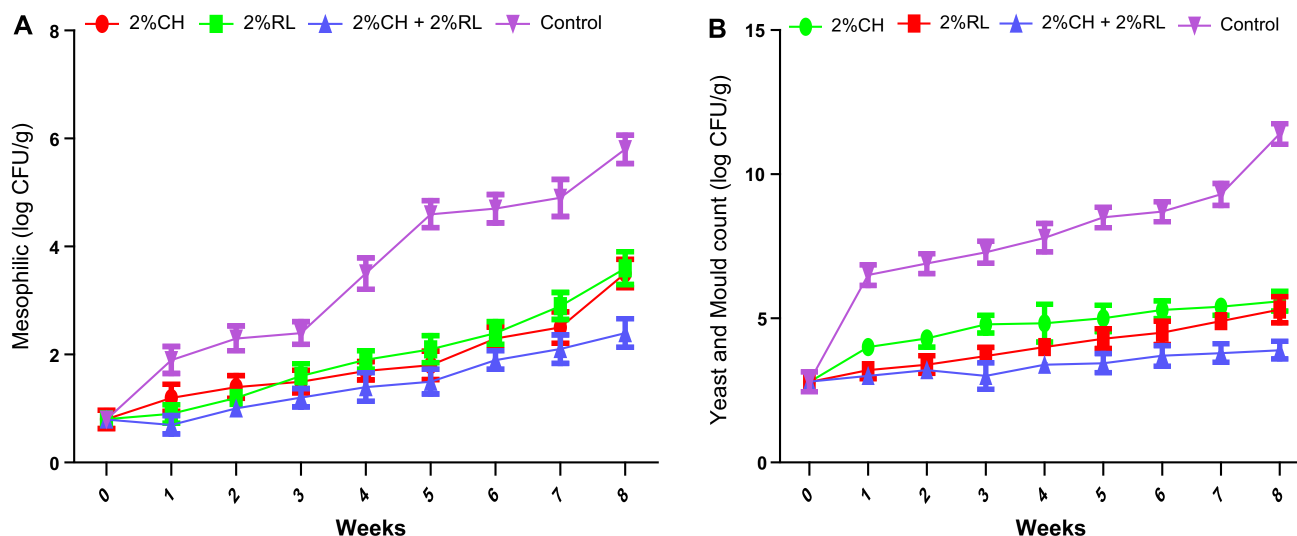

Fig. 3 Effect of chitosan and rhamnolipid coating on Mesophilic (**a**), yeast and mould count (**b**) (log CFU/g) of orange fruit during storage at 25 °C. Each value is the mean and standard errors for three replicates

development in oranges (Fig. 1C). This result is consistent with earlier reports that show that chitosan delays ripening in different fruits when used as a coating agent (Jiang and Li 2001; Ali et al. 2011). Also, some research indicates that the internal CO_2 concentrations increases in fruits coated with chitosan, and that this coating helps prevent early ripening (El Ghaouth et al. 1992; Martínez-Romero et al. 2006; Ali et al. (2011).

3.4 Malondialdehyde contents

Malondialdehyde analysis is used to measure the rate of lipid peroxidation, which is an indicator of oxidative damage or cell membrane injury under environmental stress. The T3 treatment significantly delayed ($p < 0.05$) the accumulation of malondialdehyde in coated fruits compared to the other treatment groups and the control (Fig. 1D). Malondialdehyde content in orange pulp also increased with storage time, but was higher ($p < 0.05$) in uncoated orange fruits during the 8 weeks of storage compared to the coated fruit groups. However, orange fruits in the T3 group showed an overall reduction in malondialdehyde content with storage time, followed by T1, and then T2 when compared to the control. Therefore, these results show that the combined effect of both coating factors maintained the membrane integrity of treated oranges (Fig. 1D). Malondialdehyde is a marker of lipid peroxidation; malondialdehyde has been used to demonstrate the level of oxidative damage during oxidative stress (El-Ashmawy et al. 2006). The result obtains during this study (Fig. 1D) showed that the treatment groups that included chitosan lowered malondialdehyde content and preserved the membrane integrity in coated fruits (Hong et al. 2012; Xing et al. 2011).

3.5 Antioxidant enzyme assay

CAT activities were significantly decreased ($p < 0.05$) in both coated and uncoated oranges during storage compared to their activities in oranges prior to storage (Fig. 2A).

The SOD activity in orange fruits significantly increased ($p < 0.05$) in all treatment groups after the third week of storage. The level of $O_2^{\bullet-}$ and POD activity generally increased in all groups during the storage period. This increase in POD activity was reduced ($p < 0.05$) in oranges in the T3 group beginning from the second week of storage (Fig. 2B and D). CAT has been identified as an antioxidant enzyme that plays a vital role in oxidation resistant activities in plants. The orange fruits from the T3 group showed higher CAT activity when compared to the control, suggesting that senescence of the coated orange fruits was delayed (Fig. 2A). Reactive oxygen species (ROS) are produced in cells in abiotic and biotic stress conditions (Caverzan et al. 2016). Several enzymes including SOD, CAT and POD are involved in the reduction of the reactive oxygen species, especially CAT (Lamb and Dixon 1997). Additionally, SOD participates in the rapid conversion of dismutating superoxide anion ($O_2^{\bullet-}$) into H_2O_2 in order to protect the cell from oxidative stress. In this regard, the application of chitosan to fruits as an edible coating material has been showed to promote and enhance antioxidant properties in the fruits (Cao et al. 2009; Liu et al. 2007; Dang et al. 2010; Zeng et al. 2010). In this study, we establish that the activity of SOD, CAT and POD enzymes in orange fruit coated with chitosan and rhamnolipids alone

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Table 1 Effect of chitosan–rhamnolipid concentrations on quality attributes orange fruit stored at 25 °C for 8 weeks

Attributes	Treatments	Weeks								
		0	1	2	3	4	5	6	7	8
Ascorbic acid (mg/100 g)	2% CH	158.0 ± 3.2^{ns}	143.0 ± 1.3^{ns}	123.0 ± 2.1^{b}	118.0 ± 1.4^{b}	116.0 ± 2.3^{b}	114.0 ± 1.3^{a}	109.0 ± 3.1^{a}	103.0 ± 0.8^{b}	98.0 ± 1.6^{b}
	2%RL	158.0 ± 1.2^{ns}	143.0 ± 3.4^{ns}	120.0 ± 0.7^{b}	114.0 ± 2.7^{b}	108.0 ± 1.8^{c}	105.0 ± 0.9^{b}	101.0 ± 2.5^{b}	94.0 ± 1.6^{c}	92.0 ± 1.8^{b}
	2%CH + 2% RL	158.0 ± 0.9^{ns}	143.0 ± 2.2^{ns}	135.3 ± 2.6^{a}	128.0 ± 3.2^{a}	125.0 ± 1.8^{a}	118.0 ± 1.3^{a}	114.0 ± 2.1^{a}	112.0 ± 0.5^{a}	110.0 ± 1.6^{a}
	Control	158.0 ± 2.6^{ns}	143.0 ± 3.6^{ns}	113.0 ± 2.4^{c}	104.0 ± 1.7^{c}	100.0 ± 2.1^{d}	93.0 ± 1.5^{c}	85.0 ± 1.5^{c}	78.0 ± 2.3^{d}	72.0 ± 1.6^{c}
SSC (%)	2% CH	11.6 ± 0.9^{ns}	10.3 ± 0.9^{ns}	10.4 ± 0.5^{ns}	10.5 ± 1.1^{ns}	10.6 ± 0.7^{b}	10.6 ± 1.0^{b}	10.7 ± 0.4^{b}	10.8 ± 1.2^{b}	10.9 ± 1.0^{b}
	2%RL	11.6 ± 1.1^{ns}	10.3 ± 0.6^{ns}	10.3 ± 0.8^{ns}	10.4 ± 1.1^{ns}	10.4 ± 0.3^{b}	10.5 ± 1.3^{b}	10.5 ± 0.7^{b}	10.6 ± 1.1^{b}	10.7 ± 0.6^{b}
	2%CH + 2% RL	11.6 ± 2.2^{ns}	10.3 ± 1.1^{ns}	10.3 ± 0.8^{ns}	10.3 ± 0.6^{ns}	10.3 ± 0.2^{b}	10.4 ± 0.8^{b}	10.4 ± 0.5^{b}	10.5 ± 0.9^{b}	10.5 ± 1.0^{b}
	Control	11.6 ± 1.1^{ns}	10.3 ± 0.2^{ns}	10.5 ± 0.2^{ns}	10.7 ± 1.3^{ns}	11.2 ± 0.7^{a}	11.4 ± 0.5^{a}	11.73 ± 1.2^{a}	11.9 ± 0.6^{a}	12.0 ± 1.1^{a}
Titratable acidity (%)	2% CH	0.56 ± 0.2^{ns}	0.45 ± 0.1^{ns}	0.40 ± 0.1^{b}	0.39 ± 0.3^{ns}	0.39 ± 0.2^{c}	0.37 ± 0.1^{b}	0.36 ± 0.2^{b}	0.35 ± 0.1^{a}	0.35 ± 0.1^{a}
	2%RL	0.56 ± 0.3^{ns}	0.45 ± 0.2^{ns}	0.45 ± 0.1^{a}	0.43 ± 0.1^{ns}	0.42 ± 0.2^{b}	0.41 ± 0.1^{a}	0.40 ± 0.3^{a}	0.39 ± 0.1^{b}	0.38 ± 0.1^{a}
	2%CH + 2% RL	0.56 ± 0.1^{ns}	0.45 ± 0.2^{ns}	0.45 ± 0.2^{a}	0.45 ± 0.1^{ns}	0.45 ± 0.3^{a}	0.43 ± 0.2^{a}	0.42 ± 0.1^{a}	0.41 ± 0.1^{a}	0.4 ± 0.2^{a}
	Control	0.56 ± 0.1^{ns}	0.45 ± 0.1^{ns}	0.45 ± 0.1^{a}	0.43 ± 0.2^{ns}	0.39 ± 0.1^{c}	0.37 ± 0.1^{b}	0.29 ± 0.2^{c}	0.27 ± 0.1^{d}	0.25 ± 0.1^{b}

Means within the same column with different superscripts are significantly ($p < 0.05$) different. ns = not-significant, RL = Rhamnolipid, CH = Chitosan, 2% CH + 2% RL = 2% Rhamnolipid mixed with 2% Chitosan, (mg/100 g) = mg ascorbic acid/100 g

or in combination were induced during postharvest storage (Fig. 2A, C, D).

3.6 Microbial count

All the coating treatments we tested significantly reduced ($p < 0.05$) microbial growth in the oranges during storage. Coating treatment T3 significantly reduced ($p < 0.05$) bacteria, yeast and mould populations during the storage period. After the first week, the initial mesophilic bacteria load was 1.2 log CFU $\times$ g^{-1}; and the yeast and mould count was 3.2 log CFU $\times$ g^{-1}. Oranges coated with T3, T1 and T2 treatments reduced the growth of mesophilic bacteria to 3.6 log CFU $\times$ g^{-1}, 3.5 log CFU $\times$ g^{-1} and 3.2 log CFU $\times$ g^{-1}, respectively, when compared to the control, which increased to 5.8 log CFU $\times$ g^{-1} after 8 we of storage. Moreover, oranges coated with T3, T1, and T2 treatments had reduced yeast and mould counts of 5.6 log CFU $\times$ g^{-1}, 5.3 log CFU $\times$ g^{-1} and 5.0 log CFU $\times$ g^{-1}, respectively, when compared to the control (11.4 log CFU $\times$ g^{-1}) after 8 w of storage (Fig. 3A-B). Alvarez et al. (2013) and Pushkala et al. (2012) demonstrated that chitosan contains inhibitory substances that could prevent fruit and vegetable spoilage. In this study, the synergetic effect of chitosan and rhamnolipids reduced the microbial count to a minimal level in postharvest oranges. Furthermore, the combination of chitosan and *Aloe vera* gel has a greater inhibitory effect on spoilage microorganisms from blueberry fruits (Vieira et al. 2016). The high antibacterial activity in chitosan could cause cellular disruption and consequently death of spoilage microorganisms (Cadogan et al. 2014). The crude rhamnolipids used for this study consisted of both monorhamnolipid and dirhamnolipids which might be responsible for the high antimicrobial activity observed on mesophilic bacteria, yeast and mold isolated from the oranges during storage. Similarly, Adetunji et al. (2017b) observed that the ratio of monorhamnolipid (MRL) and dirhamnolipid (DRL) congeners contained in rhamnolipids from *Pseudomonas* sp. C1501 also have antimicrobial activity against the tested pathogens (*Aspergillus flavus, Saccharomyces cerevisiae, Aspergillus niger*) isolated from wheat, onion, and banana respectively.

3.7 Quality attributes of the oranges

Application of edible fruit coatings that contained 2% chitosan, 2% rhamnolipid and 2% chitosan + 2% rhamnolipid were significantly ($p < 0.05$) higher in ascorbic acid content. However, the soluble solids content values for T1, T2 and T3 treated oranges were significantly lower ($p < 0.05$) after 4–8 w of storage than the control oranges. Coating oranges with T2 alone and T3 significantly increased ($p < 0.05$) the titratable acidity content of the fruits between 6 and 8 w of storage

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Table 2 Taste panel of oranges covered with different edible coating from chitosan and rhamnolipid during ambient storage at 25 °C

Treatment	Weeks	Appearance	Aroma	Texture	Sweetness	Acidity	Flavour	Overall liking
Control	0	5.2 ± 0.3^a	4.3 ± 0.2^a	4.9 ± 0.1^a	5.4 ± 0.3^a	5.1 ± 0.2^a	5.8 ± 0.6^a	4.9 ± 0.5^a
	4	4.0 ± 0.5^b	3.2 ± 0.1^b	3.5 ± 0.8^b	3.2 ± 0.3^b	3.0 ± 0.1^b	3.4 ± 0.3^b	2.4 ± 0.4^b
	8	2.9 ± 0.6^c	2.0 ± 0.4^c	2.2 ± 0.5^c	2.3 ± 0.1^c	3.1 ± 0.1^c	2.1 ± 0.5^c	1.9 ± 0.2^c
2% CH	0	5.2 ± 0.2^a	4.3 ± 0.1^a	4.9 ± 0.6^a	5.4 ± 1.0^a	5.1 ± 0.4^a	5.8 ± 0.2^a	4.9 ± 1.0^a
	4	4.0 ± 0.3^b	3.1 ± 0.2^b	4.3 ± 0.6^a	3.1 ± 0.3^b	3.2 ± 0.5^b	4.6 ± 1.1^b	$4.5 \pm 0.4a^b$
	8	3.8 ± 0.3^b	2.9 ± 0.1^b	3.4 ± 0.2^b	2.8 ± 0.3^b	2.9 ± 0.4^c	3.4 ± 0.3^c	4.0 ± 0.5^b
2% RL	0	5.2 ± 0.2^a	4.3 ± 0.6^a	4.9 ± 0.4^a	5.4 ± 1.1^a	5.1 ± 1.2^a	5.8 ± 0.4^a	4.9 ± 1.1^a
	4	5.0 ± 0.7^a	4.0 ± 0.8^a	4.5 ± 0.3^a	4.2 ± 0.3^b	4.0 ± 0.3^b	4.5 ± 0.8^b	3.8 ± 0.3^b
	8	3.9 ± 0.4^b	2.9 ± 0.6^b	3.8 ± 0.9^b	3.5 ± 0.3^b	3.6 ± 1.0^c	2.6 ± 0.5^c	3.5 ± 0.2^b
2%CH2%RL	0	5.2 ± 1.1^a	4.3 ± 0.2^a	4.8 ± 0.2^a	5.4 ± 0.3^a	5.1 ± 0.3^a	5.8 ± 0.4^a	4.9 ± 0.3^a
	4	5.0 ± 0.8^a	4.0 ± 0.5^a	4.3 ± 0.8^a	5.0 ± 1.3^a	5.0 ± 0.3^a	5.3 ± 0.4^b	4.3 ± 0.1^a
	8	4.5 ± 0.2^a	3.5 ± 0.4^a	4.0 ± 0.2^a	4.5 ± 0.6^a	4.5 ± 0.2^b	4.9 ± 0.6^c	4.0 ± 0.3^a

Means within the same column with different superscripts are significantly ($p < 0.05$) different. RL = Rhamnolipid, CH = Chitosan, 2% CH + 2% RL = 2% Rhamnolipid mixed with 2% Chitosan

(Table 1). Chitosan has been shown to induce reactive oxygen compounds that can be recognized by antioxidant compounds (Hodges et al. 1996). The result obtained during this study (Table 1) showed that the treated oranges prevented ascorbic acid degradation, which is consistent with earlier evidence that chitosan coatings can prevent ascorbic acid degradation on mango slices (Chien et al. 2007b). SSCs were preserved in fruits in all the treatment groups during the 8 w storage period (Table 1). This effect could be linked to the chitosan and rhamnolipid coating application, which able to modify the internal atmosphere of coated fruits (Martínez-Romero et al. 2006). These compounds can also minimize the level of gas exchange in coated fruits (Arowora et al. 2013), and chitosan had been reported to influence the level of titratable acidity, which increases the rate of senescence, and thereby effectively delays fruit ripening (Han et al. 2004). The results obtained from our study is similar to that of Han et al. (2004), who showed that chitosan could minimise the level of acidity in coated fruit during storage. Moreover, synergetic effect between chitosan and rhamnilipid used as edible coatings during this study, influenced the level of titratable acidity compared to when used singly during the 8 w of storage (Chien et al. 2007a; Nehal et al. 2012; Mohammed et al. 2016).

3.8 Sensory evaluations of the oranges

A taste panel evaluated oranges treated with different edible coatings of chitosan and rhamnolipid stored at 25 °C (Table 2) for sensory values of appearance, aroma, texture, sweetness and overall preference. These values remained unchanged in oranges that were coated with the T3 treatment. Appearance, aroma sweetness, acidity, and flavour values were reduced in oranges treated with T1 alone after the third week of storage; however, this did not have any influence on the overall preference for the T1 treated fruits. The sensory attributes appearance, aroma, and texture of the T2 coated oranges were significantly increased ($p < 0.05$), while the other sensory attributes sweetness, acidity flavour and overall preference, significantly decreased towards the end of the 8-week storage period (Table 2). The results obtained from the sensory test showed that the treated oranges were more acceptable after the 8 weeks of storage than the uncoated oranges. The combination effect of chitosan with rhamnolipid enhanced the postharvest maintenance of the oranges during storage (Table 2), which might be linked to the antimicrobial properties in the coating material that prevents the negative attributes associated with the sensory properties tested (Azarakhsh et al. 2014; Perdones et al. 2012).

In summary, our study showed that sweet orange fruits treated with chitosan and rhamnolipid coatings alone or in combination had higher psychological, biochemical, antioxidant enzyme activities, as well as a significant inhibitory effect against spoilage microorganisms. Therefore, the edible coating formulated from a mixture of chitosan and rhamnolipid may serve as an alternative to chemical-based coatings used for the prevention of postharvest loss in fruit quality. Moreover, the combinatory effect of chitosan and rhamnolipid retained the distinctive sensory attributes of the sweet orange fruits, which were reflected in overall sensory preference for the treated fruits over the control (Table 2). Further study will be needed to understand the mechanism and mode of action of chitosan and rhamnolipid in preventing or inhibiting spoilage microorganisms at molecular levels. Therefore, this study contributes to the development of indigenous wax emulsions/coatings that prolong the

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postharvest qualities of fruits, and supports the food safety objectives of the Sustainable Development Goals (SDGs).

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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