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EFFECT OF CHITOSAN–OLIVE OIL PROCESSING RESIDUES COATINGS ON KEEPING QUALITY OF COLD-STORAGE STRAWBERRY (*FRAGARIA ANANASSA*. VAR. FESTIVAL)

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ABSTRACT

Strawberry fruits have a very short shelf-life owing to their high degree of perishability. This study aimed to determine the efficacy of chitosan (CH) incorporated with olive oil residues (OOR) coatings on overall quality of cold-stored strawberry fruits compared to watery wax incorporated with thiabendazole. Strawberries were sprayed after infected with *Rhizopus stolonifer* with six different coating formulas and uncoated fruits. Indeed, all freshness and microbiological parameters were significantly increased in uncoated fruits compared to coated fruits. Amazingly, the coated strawberry using CH-OOR 2% was reduced significantly the gradual decline in their contents of total phenolics, flavonoids and their antioxidants. Likewise, it was the lowest fruits in decayed area, fungal count and malondialdehyde development. Then, fruits were coated with CH-OOR showed uniform coating distribution, since it was impossible to see any pores. Therefore, coating treatments with CH-OOR was improved the membrane integrity and increased the keeping quality.

PRACTICAL APPLICATIONS

Edible coatings could be an effective way for delaying the ripening process and extending the shelf-life stability of strawberry during postharvest. The effectiveness of chitosan (CH)-olive oil residue (OOR) as a novel edible coating, in comparison with watery wax-thiabendazole was approved. The applicability of those films to maintain microbiological and freshness quality in strawberry during postharvest was succeed. Incorporation of OOR into CH increased its antifungal property against *R. stolonifer in vivo* and in *vitro*. Coating by CH-OOR reduced phenolics, flavonoids and antioxidants decomposition in coated fruits compared to uncoated. CH-OOR was able to slow down the gas exchange by reducing the CO_2 of coated strawberry, which reduced their malondialdehyde development. Commercially, CH-OOR could be explored as a novel and potential natural coating to substitute the synthetic agents in fruit packaging industries. The coating cost could increase the total cost about 6–8% where shelf-life and keeping quality was improved.

INTRODUCTION

Egypt is ranked globally as the first olive production in quantity per hectare to be 97.88 Hg/Ha (Fao 2013a). Surprisingly, a large amount of total phenolic compound (TPC), antioxidant activity (AOA) and antimicrobials agents can be extracted from olive oil residues (OOR) (Lafka *et al.* 2011; Brahmi *et al.* 2012; Keskın *et al.* 2012; Terpinc *et al.* 2012; Esteve *et al.* 2015). Thus, it may be incorporated with edible films (Özge *et al.* 2013). Afterwards, Egypt was contributed by 5.5% in the global production of strawberry to be 242.297 tons, occupies the fifth production country worldwide (Fao 2013b). It's unique, highly desirable aroma

and phytochemicals (Van De Velde *et al.* 2013). The pathogenic microorganisms may be growth in fruit's surface during postharvest. Thus, it can be promote decay, produce mycotoxins and degraded phytochemicals (Matthes and Schmitz-Eiberger 2012). Commonly, these challenges might be fixed using coating by commercial wax incorporated with some additives such as thiabendazole (TBZ). However, it caused some dangerous side effects (List 2005). Therefore, modern trend by using some natural polymers such as chitosan (CH) incorporated with natural additives was recently discussed.

CH (poly B-(1,4) *N*-acetyl-D-glucosamine) is linear polysaccharides, derived from exoskeleton of invertebrates by deacetylation of chitin (Meng *et al.* 2008). It is the second most abundant polysaccharide after cellulose (Martínez-Camacho *et al.* 2010). It is a very promising biopolymer because it's environmental friendly, good film-forming properties (Aider 2010), good mechanical properties included selective permeability to gases (CO_2/O_2) (Coma 2008), antibacterial activity (Bordenave *et al.* 2007; Bourtoom and Chinnan 2008; Ziani *et al.* 2008), antifungal activity (Fernandez-Saiz *et al.* 2009; Aider 2010; Ojagh *et al.* 2010b), AOA (Ben-Shalom and Fallik 2003) and produced from renewable sources with low cost (Conte *et al.* 2013). Surely, it's harmless to humans (Ojagh *et al.* 2010a), nontoxic (Han *et al.* 2004; Ribeiro *et al.* 2007), GRAS (Kean and Thanou 2010) and approved by USFDA (Jayakumar *et al.* 2005; Prabaharan and Mano 2006). The plant extracts are safe, natural and classified as GRAS in food industry (Ayana and Turhan 2009). Therefore, food residues extracts can be incorporated with CH to control the mold fungal and keep the fruits quality such as in citrus (Shao *et al.* 2015), blueberries (Yang *et al.* 2014), strawberries (Perdones *et al.* 2014; Yang *et al.* 2014).

However, to our knowledge, there is no scientific literature available regarding the effect of OOR incorporation with antifungal CH coatings on the postharvest quality characteristics of strawberry fruits. Therefore, the present study has been undertaken with the objective of elucidating the potential of CH only or combinations with OOR on shelf-life extension and quality improvement of cold-stored strawberry fruit comparing with commercial waxing watery wax (WW)-TBZ.

MATERIALS AND METHODS

Reagents and Solutions

1, 1-diphenyl-2 picrylhydrazyl radical ($DPPH^{\bullet}$), 2-(3, 4-dihydroxyphenyl)−3, 5, 7-trihydroxy-4H-chromen-4-one (Quercetin) and 6-hydroxy-2,5,7,8-tetramethylchroman carboxylic acid (Trolox) were obtained from Sigma Aldrich, Germany. 2, 6-dichlorophenol-indophenol and Folin–Ciocalteu reagent were obtained from Fluka Biochemical, Switzerland. Gallic acid, Serva, Biochemical, New York. Chitosan 95% deacetylation, Oxford, India. Thiabendazole (TBZ) and water wax (WW), Fomesa Fruitech, S.L., Spain.

Microbial Strains and Media

Rhizopus stolonifer ATCC 14037 was obtained from Cairo Microbiological Resource Center (MIRCEN), Fac. of Agric., Ain Shams Univ., Cairo, Egypt. Rose bingle agar No. 401992 and Sabouraud agar No. 402005 were obtained from Biolife, Italy.

Raw Materials

Olive (*Olea. europaea* var. *Kronakii*) residues included olive leaves and olive pomace from the crop of summer session 2012 was obtained from the Cairo for oil industry, industrial zone, 6th October city, Egypt.

Fresh strawberry fruits (*Fragaria ananassa.* var. *Festival*) were obtained from Abo-Rahia farm, Toukh city, Egypt.

Methods

Olive Processing Residues Preparation and Extraction. The obtained olive residues in fresh status were transferred immediately to the analytical lab. Both residues were dried by oven dryer (Tit Axon S.R.L via Canova, Italy) at 40–50C gradually for 12 h. Subsequently, these were milled by grinder (Severin, type 3871, Germany) and passed through a 60 mesh sieve to obtain fine homogenous powder. Then they were immediately packed in dark glass jars then kept at -18 ± 1C until use.

Conversely, both OOR were mixed with ethanol 80% as (1:20, w/v) individually in dark bottles with shaking at 120 rpm for 24 h. The mixture were filtered through filter paper Whatman No.1 and the filtrates were collected, then solvents were removed by rotary evaporator (NE-1-Rikakikai Co., LTD, Japan) at 40C. The obtained extracts after evaporation were lyophilized (CHRIST, ALPHA 1-4D plus, Germany) then kept at $-18 + 1$C until further uses according to (Lafka *et al.* 2011; Mohagheghi *et al.* 2012).

Culture Propagation. The *R. stolonifer* was inoculated on Sabouraud agar and incubated at 28C for 5 days. Fungal spore suspension was prepared by washing the 5-old day's cultures using 10 mL tween 80 solution 10 mL/L using glass rod to make a stock suspension solution. Spores content by Thoma's cell with light microscopy were counted to calculate the inoculums volume.

Film Formation. The incorporated CH films with freeze-dried ethanolic olive leaf extracts (OLE) and ethanolic olive pomace extracts (OPE) were prepared according to Gol *et al.* (2013) with some modifications. A 20 g/L CH was dispersed in an aqueous solution of glacial acetic acid (0.5%, v/v) at

40C. The solution was heated and agitated constantly for 12 h then the pH was adjusted to 5.6 with 1 N NaOH. Subsequently, glycerol 1.6% was added as a plasticizer (Sánchez-González *et al.* 2011). The solution was stirred overnight at room temperature (25 ± 3C), then the OLE and OPE were added to reach a final concentration of 1 and 2% (v/v). CH-OLE and CH-OPE were mixed to achieve complete dispersion.

Strawberry Fruits Coating Treatments. Strawberry fruits were sorted for uniform size, color, maturity and for being free of visible defect as well as decay. The fruits were sanitized by sodium hypochlorite solution (250 ppm) and then washed by distilled water. The coating solutions (as described above) were sprayed on the whole strawberries' surface using a Multi-function hand 2L pressure sprayer (Ningbo Synkemi. Co., type SK-2B, China) in twice (∼60 s) and air-dried at room temperature for 2 h. Seven groups of samples were prepared in total uncoated (control), coated with CH (2% w/v), coated with CH-OLE (1% w/v), coated with CH-OLE (2% w/v), coated with CH-OPE (1% w/v), coated with CH-OPE (2%w/v), coated with WW-TBZ 0.1% as positive control according to Zhang and Quantick (1997). Fruits were packed in boxes [∼3 fruit (80 ± 5 g) per box] and warped with polyethylene sheets, then stored at 4 ± 1C for 16 days. The quality attributes of uncoated and coated fruits were evaluated at the beginning of the experiment (i.e., 0 days) and after 4, 8, 12, 16 days.

Quality Attributes of Coated Strawberry Fruits. ***Weight Losses.*** The weight of fruits was recorded at the beginning of the experiment (i.e., 0 day) and every 4 days. The difference between the initial weight and each weight of the fruits was considered as a total weight loss. The results were expressed as percentage weight loss according to AOAC (2000).

Determination of Total Soluble Solids, Titratable Acidity and Total Sugar. After remove the decayed area from all samples, the strawberry puree was prepared using Hemisphere fruit juice machine multifunctional ALIEXPRESS, Co., China, then it was used to evaluate the different quality parameters. The total soluble solids (TSS) and total sugar (TS) was determined according to AOAC (2000). The titratable acidity (TA) was determined according to Bassetto *et al.* (2005). The results were expressed as citric acid equivalent.

Ascorbic Acid. The ascorbic acid (AA) content in strawberry fruits during storage periods were determined by using 2,6-dichlorophenol-indophenol titrimetric method according to Thimmaiah (1999). A pure AA was used to prepare a standard solution (1 mg/mL). The AA content was expressed as mg/100 g, dw.

Anthocyanin Content. The anthocyanin content of strawberry fruits were analyzed according to Fuleki and Francis (1968) with some modification. A 5 g samples were extracted with 45 mL of acidified ethanol (95% ethanol: HCl 1.5N 85:15) for 2 h in the dark then filtered through Whatman No.1 filter paper. Absorbance was measured at 535 nm; the results were expressed as mg/100 g, dw.

Total Phenolics Content. The TPC for acetone extracts of strawberry were determined according to Pineli *et al.* (2011). In brief, 200 μL of each extract was mixed with 1 mL of 10-fold diluted Folin–Ciocalteu reagent, after 5 min the reaction was stopped by 1 mL of 7.5% Na_2CO_3 then 1.5 mL distilled water was added. The mixture was incubated in dark for 60 min then the absorbance at 760 nm was measured. The TPC was expressed as milligrams of gallic acid equivalents (GAE) (mg of GAE/100 g, dw) using the following equation:

$$Y = 0.0201\ x + 0.0538 \quad (R^2 = 0.99) \tag{1}$$

where: Y is the concentration and x is the absorbance.

Total Flavonoids. Total flavonoids content (TF) for acetone extracts of strawberry was determined according to Mohdaly *et al.* (2012). A 0.5 mL aliquot of 2% $AlCl_3$ ethanolic solution was added to 0.5 mL of extracts and mixed well then kept for 1 h and the absorbance at 420 nm was measured. The final concentration of TF was expressed as quercetin equivalent (QE) (mg QE/g, dw) which was calculated using the following equation based on the calibration curve:

$$Y = 0.037x + 0.1363 \quad (R^2 = 0.98) \tag{2}$$

where: Y is the concentration and x is the absorbance.

Antioxidant Activity. The AOA of acetone extracts of strawberry fruits were evaluated according to Pineli *et al.* (2011). A 0.1 mL from extract was added to 3.9 mL of $DPPH^{\bullet}$ methanolic solution (0.0025 g/100 mL) then the absorbance at 517 nm was measured after the solution had been allowed to stand in the dark for 60 min and measured at 517 nm. The final results were expressed as micromoles of Trolox equivalents (TE) per gram of dry weight (μmol TE/g, dw).

Determination of Deterioration of Strawberries Tissue. Malondialdehyde (MDA) contents were measured according to Hong *et al.* (2012). Fruits pulp (2.0 g) from each sample individually was homogenized in 6 mL of 10% trichloroacetic acid and then centrifuged for 15 min at 6,000 × g. The supernatant phase was collected and 2 mL was mixed with 6 mL of 0.6% thiobarbituric acid. The mixture was heated up to 100C for 20 min, quickly cooled down and centrifuged at 6,000 × g for 10 min. The supernatant was collected and absorbance was measured at 450, 532 and 600 nm. The MDA concentration was calculated according to the following equation:

$$MDA = [(6.45 \times (A_{532} - A_{600})) - (0.56 \times A_{450})] \quad (3)$$

where: A_{532}: Absorbance at 532 nm, A_{600}: Absorbance at 600 nm, A_{450}: Absorbance at 450 nm and 6.45 as well 0.56 are a consonant.

Microstructure Analysis. The surface and cross-section microstructure of strawberry skins which coated by CH film, CH-OLE (2%), CH-OPE (2%) and uncoated fruits were examined under a scanning electron microscope (SEM). Tissues from fruits were fixed in 4% glutaradehyde in 0.2 M sodium cocodylate buffer (pH 4.1) for 4 h, then fixated later in osmium tetraoxide for 2 h. Fixed tissues were rinsed in same puffer three times and dehydrated through a graded ethanol series 10 to 100% for 10 min ended by 30 min in final concentration. The specimens were transferred on cupper slide and dehydrated using critical point dryer with liquid carbon dioxide, then coated with gold using (S150A Sputter coater-Edwards-England). Finally, the specimens were examined and photographed using SME (JXA-840A, Electron Probe Micro analyzer-JEOL, Japan).

Effect of Coating on the Growth of R. Stolonifer Fungal Strains. The effect of CH-film, CH-OOR-incorporated films and WW-TBZ were evaluated *in vivo* against *R. stolonifer* according to Maqbool *et al.* (2010) with minor modifications. The strawberry fruits was disinfected with sodium hypochlorite (250 ppm) for 2 min, then rinsed in sterilized water for 2 min and left until they dried completely. Subsequently, cross-shaped wounds were made on fruit using sterilized puncher. Immediately, strawberry fruits were inoculated similarly by *R. stolonifer* spores suspension (10^5 spores/mL). After 15 min, the inoculated fruits were coated by prepared coating solutions. Uncoated and coated fruits were stored at 4 ± 1C for 16 days. Mold growth of inoculated fruits was checked each 4 days by measuring of the decay area by Standard Gage Vernier Calipers (microntesa, Co., South Africa) and follow up the fungal count.

Statistical Analysis

The statistical analysis was carried out using SPSS program (ver. 19) with multifunction utility regarding to the experimental design under significance level of 0.05 for the whole results and multiple comparisons were carried out applying LSD according to Steel *et al.* (1997).

RESULTS AND DISCUSSION

Freshness Parameters of Strawberry Fruits during Cold Storage

Weight Loss. The effect of CH-OOR films on weight loss in strawberry fruits during cold storage was investigated; data was shown in Table 1. The weight loss increased during a prolonged storage period. Surely, coating treatments reduced significantly ($P < 0.05$) the weight loss compared with uncoated fruits. Obviously, significant difference ($P < 0.05$) was found between CH incorporated film and TBZ coating. The lowest weight loss in strawberry at the end of storage period was 5.64% using CH-OLE 1%. The formed CH film on surface of coated fruits delayed the migration of moisture. These coating strategies to reduce the weight loss during storage were used in strawberries as recently mentioned (Gol *et al.* 2013).

Total Soluble Solids. The TSS of both uncoated and coated strawberry fruits during cold storage was represented in the same table. Clearly, TSS level at the beginning of storage was 5.30%. They are increased at the end of storage in both uncoated and coated fruits. The increasing in TSS during storage was mentioned by Rivera-López *et al.* (2005), due to the breakdown of starch into soluble sugars or the hydrolysis of cell wall's polysaccharides (Crouch 2003).Controversial, uncoated fruits exhibited TSS higher than coated fruits with CH or CH-OOR reached to 8.43%. Obviously, the TSS contents increased quickly in uncoated fruits, conversely increased regularly in all coated fruits. Conversely, coated strawberry with CH-OPE 2% recorded the lowest incremental rate in TSS reached to 6.53%. Our results assured that the filmogenic property of CH or CH-OOR exudes efficient permeable films for vegetables coating. It may seem to work as pores barrier reducing O_2 exchange. Also they may suppressing ethylene evolution process (Dong *et al.* 2004). These results are lined with those mentioned by Hong *et al.* (2012) and Gol *et al.* (2013).

Total Sugars. Contrariwise, the effect of coating materials on TS in strawberry fruits during cold storage was examined; data was exposed in Table 1. Obviously, the increasing of storage period was correlated with increasing of TS. Likewise, significant difference ($P < 0.05$) was observed between coating and uncoated fruits. However, no significant difference ($P > 0.05$) was obtained between coated fruits with CH-OLE 2% and CH-OPE 2% or between coated fruits with CH, CH-OLE 1% and CH-OPE 1%. Undoubtedly, the uncoated fruits recorded the highest fruits in contents of TS at the end of storage period compared with coated fruits. Then, the coated strawberry with CH-OPE 2% and CH-OLE2% scored the lowest TS contents. The conversion of starch to soluble sugars is continuing during storage (Beaudry *et al.* 1989), resulting in increase of TS as storage period was elongated (Crouch 2003). Also, Velickova *et al.* (2013) suggested that coating of strawberry using CH improved the reduction rate in TS very low compared with uncoated strawberry.

Titratable Acidity. The effect of coating on the TA of strawberry fruits was shown in the same table. The initial TA

TABLE 1. EFFECT OF CHITOSAN (CH) AND CH-BASED FILM WITH OLIVE OIL RESIDUE (OOR) ON FRESHNESS PARAMETERS OF STRAWBERRY FRUITS DURING COLD STORAGE AT 4 ± 1C (MEAN ± SD)

	Freshness parameters					
Treatments	Storage period (day)	Weight loss (%)	Total soluble solid °Brix	Total sugar (%)	Titerable acidity (%)	MDA (mmol g^{-1} fw)
Un coated	0	–	5.30 ± 0.15^{aA}	4.78 ± 0.20^{aB}	0.60 ± 0.03^{eE}	2.55 ± 0.11^{aA}
	4	4.72 ± 0.73^{fA}	6.23 ± 0.15^{dB}	4.16 ± 0.80^{aA}	0.48 ± 0.02^{dD}	3.11 ± 0.09^{dB}
	8	7.09 ± 0.26^{fB}	7.60 ± 0.10^{dC}	5.97 ± 0.21^{eC}	0.32 ± 0.02^{cC}	3.90 ± 0.12^{eC}
	12	10.42 ± 0.82^{gC}	7.97 ± 0.15^{fD}	7.00 ± 0.16^{fD}	0.23 ± 0.01^{AB}	6.12 ± 0.03^{fD}
	16	14.70 ± 1.13^{gD}	8.43 ± 0.15^{fE}	7.51 ± 0.22^{fE}	0.17 ± 0.01^{aA}	11.95 ± 0.44^{gE}
CH	0	–	5.30 ± 0.15^{aA}	4.78 ± 0.20^{aB}	0.60 ± 0.03^{aD}	2.55 ± 0.11^{aA}
	4	3.89 ± 0.89^{dA}	5.90 ± 0.10^{cA}	4.29 ± 0.06^{bA}	0.52 ± 0.02^{bC}	2.57 ± 0.01^{aA}
	8	4.24 ± 0.93^{dA}	6.73 ± 0.15^{dB}	5.23 ± 0.17^{bcC}	0.40 ± 0.02^{bB}	2.97 ± 0.03^{cdB}
	12	6.89 ± 0.80^{eB}	7.20 ± 0.10^{eC}	5.91 ± 0.04^{dD}	0.26 ± 0.01^{bA}	4.64 ± 0.02^{dC}
	16	8.67 ± 0.63^{eC}	7.50 ± 0.10^{dC}	6.21 ± 0.03^{dE}	0.22 ± 0.05^{bA}	7.60 ± 0.04^{eD}
WW-TBZ	0	–	5.30 ± 0.15^{aA}	4.78 ± 0.20^{aB}	0.60 ± 0.03^{aD}	2.55 ± 0.11^{aA}
	4	4.33 ± 0.96^{eA}	6.03 ± 0.15^{cB}	4.28 ± 0.02^{bA}	0.53 ± 0.02^{Bc}	2.95 ± 0.06^{cB}
	8	5.47 ± 0.38^{eB}	6.70 ± 0.17^{dC}	5.58 ± 0.06^{dC}	0.33 ± 0.01^{abB}	2.45 ± 0.00^{aA}
	12	8.43 ± 0.71^{fC}	7.00 ± 0.10^{dD}	6.06 ± 0.08^{eD}	0.24 ± 0.02^{aA}	5.11 ± 0.10^{eC}
	16	9.79 ± 0.62^{fD}	7.70 ± 0.10^{eE}	6.89 ± 0.16^{eE}	0.20 ± 0.01^{bA}	8.62 ± 0.15^{fD}
CH-OLE 1%	0	–	5.30 ± 0.15^{aA}	4.78 ± 0.21^{aB}	0.60 ± 0.03^{aD}	2.55 ± 0.11^{aA}
	4	2.25 ± 0.60^{cA}	6.00 ± 0.10^{cB}	4.25 ± 0.20^{bA}	0.54 ± 0.01^{bC}	2.66 ± 0.10^{bA}
	8	2.50 ± 0.54^{aA}	6.47 ± 0.15^{cC}	5.18 ± 0.02^{bC}	0.38 ± 0.02^{bB}	2.85 ± 0.07^{cB}
	12	5.50 ± 0.56^{dB}	6.67 ± 0.15^{cC}	5.79 ± 0.03^{cD}	0.30 ± 0.02^{cA}	3.61 ± 0.05^{bC}
	16	7.28 ± 0.29^{dC}	7.20 ± 0.10^{cD}	6.05 ± 0.07^{bE}	0.27 ± 0.01^{cA}	6.31 ± 0.02^{cD}
CH-OPE 1%	0	–	5.30 ± 0.15^{aA}	4.78 ± 0.21^{aB}	0.60 ± 0.03^{aC}	2.55 ± 0.11^{aA}
	4	2.26 ± 0.65^{cA}	5.93 ± 0.12^{cB}	4.31 ± 0.17^{bA}	0.57 ± 0.02^{cB}	2.56 ± 0.09^{aA}
	8	3.14 ± 0.15^{bB}	6.00 ± 0.10^{bC}	5.17 ± 0.30^{bC}	0.33 ± 0.02^{aA}	2.86 ± 0.02^{cB}
	12	4.48 ± 1.04^{bC}	6.33 ± 0.15^{bD}	5.67 ± 0.06^{bD}	0.32 ± 0.01^{cA}	3.95 ± 0.20^{cC}
	16	5.64 ± 0.56^{aD}	7.13 ± 0.15^{cE}	6.11 ± 0.17^{cE}	0.29 ± 0.01^{cdA}	7.04 ± 0.08^{dD}
CH-OLE 2%	0	–	5.30 ± 0.15^{aA}	4.78 ± 0.21^{aB}	0.60 ± 0.03^{aE}	2.55 ± 0.11^{aA}
	4	1.87 ± 0.97^{aA}	5.70 ± 0.10^{bB}	4.29 ± 0.11^{bA}	0.54 ± 0.02^{bD}	2.50 ± 0.14^{aA}
	8	3.54 ± 0.89^{cB}	5.90 ± 0.10^{aB}	5.05 ± 0.25^{aC}	0.46 ± 0.01^{cC}	2.69 ± 0.07^{bA}
	12	4.60 ± 0.51^{cC}	6.27 ± 0.15^{bC}	5.34 ± 0.09^{aD}	0.38 ± 0.01^{dB}	3.09 ± 0.07^{aB}
	16	6.45 ± 0.64^{bD}	6.97 ± 0.12^{bD}	5.81 ± 0.11^{aE}	0.30 ± 0.02^{dA}	5.28 ± 0.10^{aC}
CH-OPE 2%	0	–	5.30 ± 0.15^{aA}	4.78 ± 0.21^{aB}	0.60 ± 0.03^{aE}	2.55 ± 0.11^{aA}
	4	2.03 ± 0.12^{bA}	5.50 ± 0.10^{aA}	4.11 ± 0.08^{aA}	0.56 ± 0.01^{cD}	2.66 ± 0.22^{bA}
	8	3.06 ± 0.62^{bB}	5.83 ± 0.15^{aB}	5.06 ± 0.10^{aC}	0.50 ± 0.01^{dC}	2.82 ± 0.05^{cB}
	12	3.87 ± 0.75^{aB}	6.03 ± 0.15^{aB}	5.38 ± 0.01^{aD}	0.39 ± 0.02^{dB}	3.16 ± 0.05^{aC}
	16	6.65 ± 0.49^{cC}	6.53 ± 0.15^{aC}	6.00 ± 0.66^{bE}	0.30 ± 0.01^{dA}	5.83 ± 0.13^{bD}

$n = 3$.

a, b, c: Means with the same letter in the same column for each formula are not significantly different ($P > 0.05$).

A, B, C: Means with the same letter in the same column for each storage period are not significantly different ($P > 0.05$).

was 0.60%; however it was decreased at the end of storage to be 0.24%. Combined CH with OLE and OPE were reduced the acidity reduction significantly compared with uncoated or coated fruits with CH only or WW-TBZ. Generally, significant difference ($P < 0.05$) was found between all treatments, except CH-OLE 2% or CH-OPE 2%, hence all of them recorded the lowest decremental rate in TA to be 0.29 and 0.30%, respectively. Also, uncoated fruits were scored the highest decreases in TA contents reached to 0.17%. For explanation, the changes in TA are significantly affected by the rate of metabolism (Clark *et al.* 2003) especially respiration, which consumed organic acids and therefore decline the acidity during storage (Maftoonazad *et al.* 2008; Ghafir *et al.* 2009), this also may cause fruit senescence (Chan *et al.* 2004). Retention of acidity decreases has been reported previously in coated strawberry fruit with CH-beeswax coatings (Gol *et al.* 2013).

Malondialdehyde. MDA content have been used as direct indicator of cell membrane injury and index of cell oxidative damage (Xu *et al.* 2009). As shown in Table 1. MDA contents in strawberry pulp increased during cold storage period. Obviously, the uncoated fruits recorded significantly higher MDA than coated fruits. To enumerate that

TABLE 2. EFFECT OF CHITOSAN (CH) AND CH-BASED FILM WITH OOR ON PHYTOCHEMICALS AND ANTIOXIDANT ACTIVITY OF STRAWBERRY FRUITS DURING COLD STORAGE AT 4 ± 1C (MEAN ± SD)

	Phytochemicals analysis					
Treatments	Storage period (day)	TPC (mg 100 g^{-1})	TF (mg 100 g^{-1})	Anthocyanin (mg 100 g^{-1})	Ascorbic acid (mg 100 g^{-1})	AOA (mg 100 g^{-1})
Un coated	0	2.03 ± 0.06^{aE}	5.51 ± 0.80^{aE}	43.69 ± 0.46^{aB}	53.58 ± 1.14^{aE}	16.69 ± 0.06^{aE}
	4	1.45 ± 0.02^{aD}	2.14 ± 0.02^{aD}	50.53 ± 1.70^{aD}	42.43 ± 1.77^{aD}	7.80 ± 0.80^{aD}
	8	0.85 ± 0.02^{aC}	1.16 ± 0.04^{aC}	60.43 ± 0.76^{aE}	30.86 ± 0.97^{aC}	6.80 ± 0.67^{aC}
	12	0.56 ± 0.01^{aB}	0.29 ± 0.03^{aB}	47.11 ± 0.48^{aC}	20.84 ± 1.40^{aB}	1.93 ± 0.71^{aB}
	16	0.23 ± 0.01^{aA}	0.10 ± 0.02^{aA}	22.3 ± 0.92^{aA}	9.27 ± 1.27^{aA}	0.65 ± 0.10^{aA}
CH	0	2.04 ± 0.06^{aE}	5.51 ± 0.80^{aE}	43.69 ± 0.46^{aA}	53.58 ± 1.14^{aE}	16.81 ± 0.04^{aE}
	4	1.80 ± 0.02^{bD}	2.37 ± 0.12^{bD}	53.10 ± 0.28^{bB}	45.46 ± 1.25^{cD}	10.89 ± 0.39^{dD}
	8	1.00 ± 0.02^{bC}	2.26 ± 0.02^{bC}	64.09 ± 1.07^{cC}	42.27 ± 1.70^{cC}	7.54 ± 1.04^{bC}
	12	0.81 ± 0.02^{bB}	1.01 ± 0.05^{bB}	72.65 ± 1.29^{cD}	33.83 ± 2.08^{cB}	4.16 ± 0.52^{cB}
	16	0.42 ± 0.01^{cA}	0.92 ± 0.02^{cA}	61.59 ± 1.20^{cC}	22.95 ± 1.40^{cA}	1.95 ± 0.40^{cA}
WW-TBZ	0	2.02 ± 0.05^{aE}	5.51 ± 0.80^{aE}	43.69 ± 0.46^{aA}	53.58 ± 1.14^{aE}	16.66 ± 0.06^{aE}
	4	1.80 ± 0.02^{bD}	2.67 ± 0.07^{dD}	53.03 ± 0.46^{bB}	43.63 ± 0.54^{bD}	9.54 ± 0.13^{cD}
	8	0.99 ± 0.02^{bC}	2.24 ± 0.06^{bC}	61.71 ± 1.25^{bC}	38.92 ± 0.87^{bC}	7.20 ± 0.94^{bC}
	12	0.75 ± 0.01^{bB}	0.98 ± 0.03^{bB}	68.86 ± 1.19^{bD}	28.31 ± 1.38^{bB}	1.84 ± 0.25^{aB}
	16	0.31 ± 0.02^{bA}	0.53 ± 0.07^{bA}	54.75 ± 0.90^{bB}	18.88 ± 1.45^{bA}	1.35 ± 0.06^{bA}
CH-OLE 1%	0	2.05 ± 0.06^{aE}	5.51 ± 0.80^{aD}	43.69 ± 0.46^{aA}	53.58 ± 1.14^{aE}	16.89 ± 0.03^{aE}
	4	1.93 ± 0.01^{cD}	2.56 ± 0.07^{cC}	53.89 ± 0.18^{bB}	47.05 ± 1.69^{dD}	8.72 ± 0.35^{bD}
	8	1.22 ± 0.02^{dC}	2.62 ± 0.07^{dC}	72.04 ± 1.02^{dC}	44.26 ± 1.00^{dC}	8.10 ± 0.63^{cC}
	12	0.99 ± 0.02^{cB}	1.31 ± 0.03^{dB}	77.72 ± 1.14^{dD}	38.29 ± 1.66^{eB}	4.35 ± 0.29^{cB}
	16	0.47 ± 0.01^{cdA}	1.04 ± 0.04^{dA}	71.73 ± 0.92^{dC}	29.90 ± 0.84^{dA}	3.20 ± 0.40^{eA}
CH-OPE 1%	0	2.04 ± 0.06^{aE}	5.51 ± 0.80^{aD}	43.69 ± 0.46^{aA}	53.58 ± 1.14^{aE}	16.8 ± 0.03^{aE}
	4	1.88 ± 0.02^{bD}	2.55 ± 0.05^{cC}	55.05 ± 0.76^{cB}	46.83 ± 1.16^{cdD}	10.22 ± 0.97^{dD}
	8	1.13 ± 0.02^{cC}	2.47 ± 0.05^{cC}	72.71 ± 0.59^{dC}	42.43 ± 0.47^{cD}	7.26 ± 0.20^{aC}
	12	0.97 ± 0.01^{cB}	1.12 ± 0.05^{cB}	83.83 ± 1.96^{eE}	36.04 ± 1.61^{dB}	3.61 ± 0.80^{bB}
	16	0.41 ± 0.01^{cA}	0.92 ± 0.04^{cdA}	79.61 ± 1.04^{eD}	28.83 ± 1.45^{dA}	2.73 ± 0.42^{dA}
CH-OLE 2%	0	2.08 ± 0.07^{aE}	5.51 ± 0.80^{aE}	43.69 ± 0.46^{aA}	53.58 ± 1.14^{aE}	17.00 ± 0.03^{aE}
	4	1.97 ± 0.02^{cD}	4.43 ± 0.22^{eD}	55.05 ± 0.28^{cB}	51.62 ± 0.81^{eD}	12.72 ± 1.21^{eD}
	8	1.38 ± 0.01^{eC}	3.93 ± 0.23^{fC}	75.09 ± 0.74^{eC}	47.29 ± 1.41^{eC}	11.78 ± 0.71^{eC}
	12	1.14 ± 0.01^{eB}	2.10 ± 0.04^{eB}	90.43 ± 1.01^{fD}	42.95 ± 0.19^{gB}	8.61 ± 0.70^{eB}
	16	0.63 ± 0.02^{fA}	1.50 ± 0.05^{fA}	89.02 ± 1.48^{fD}	35.46 ± 0.61^{fA}	6.06 ± 0.56^{gA}
CH-OPE 2%	0	2.06 ± 0.07^{aD}	5.51 ± 0.80^{aE}	43.69 ± 0.46^{aA}	53.58 ± 1.14^{aE}	16.92 ± 0.03^{aE}
	4	1.94 ± 0.00^{cC}	4.71 ± 0.31^{fD}	56.82 ± 0.37^{dB}	51.82 ± 1.65^{eD}	12.95 ± 0.78^{eD}
	8	1.26 ± 0.02^{dB}	3.67 ± 0.09^{eC}	78.70 ± 1.38^{fC}	47.36 ± 0.92^{eC}	10.83 ± 0.54^{dC}
	12	1.02 ± 0.02^{cdB}	2.05 ± 0.14^{eB}	95.32 ± 1.20^{gE}	40.09 ± 1.73^{fB}	6.61 ± 0.70^{dB}
	16	0.56 ± 0.00^{deA}	1.29 ± 0.07^{eA}	91.65 ± 0.48^{gD}	32.81 ± 1.63^{eA}	4.46 ± 0.47^{fA}

$n = 3$.

a, b, c: Means with the same letter in the same column for each formula are not significantly different ($P > 0.05$).

A, B, C: Means with the same letter in the same column for each storage period are not significantly different ($P > 0.05$).

the MDA in uncoated strawberry to be 5.52 mmol/g, fw, however, it reached in strawberry coated with CH-OLE 2% to 3.22 mmol/g, fw. Generally, strawberry were coated with CH-OLE 2% recorded the lowest incremental rate of MDA at different storage periods. The dramatically increase of MDA in uncoated strawberry, resulted soft and pale tissues. However, coating treatment was improved the membrane integrity and increased the keeping quality. Moreover, coating with Nano-packaging materials was extended the shelf-life stability of fresh strawberry fruits (Yang *et al.* 2010) and in apple (Shao *et al.* 2012).

Phytochemicals Parameters of Strawberry Fruits during Cold Storage

Total Phenolics Contents. The effect of coating on TPC contents in strawberry fruits during cold storage was studied and data was presented in Table 2. Coating treatments significantly ($P < 0.05$) decreased the TPC degradation compared with uncoated fruits. For example CH-OLE 2% recorded the lowest degradation rate of TPC. Obviously, the TPC decreased rapidly in uncoated fruits compared with coated fruits. The lowest decreases in TPC were showed in coated strawberry fruits with CH-OLE 2% to be 0.63 mg GAE/g.

TABLE 3. EFFECT OF CHITOSAN (CH) AND CH-BASED FILM WITH OOR MICROBIOLOGICAL ATTRIBUTES OF STRAWBERRY FRUITS DURING COLD STORAGE AT 4 ± 1C (MEAN ± SD)

	Microbiological parameters		
Treatments	Storage period (day)	Decayed area (mm)	*R. stolonifer* (CFU g^{-1})
Un coated	0	2.00 ± 0.0^{aA}	2.64 ± 0.39^{aA}
	4	8.50 ± 0.71^{eB}	4.10 ± 0.07^{eB}
	8	16.50 ± 0.71^{fC}	6.09 ± 0.07^{gC}
	12	20.00 ± 0.0^{fD}	10.16 ± 0.07^{fD}
	16	26.50 ± 0.71^{gE}	12.15 ± 0.03^{gE}
CH	0	2.00 ± 0.0^{aA}	2.64 ± 0.39^{aA}
	4	5.50 ± 0.71^{dB}	3.06 ± 0.06^{dA}
	8	11.50 ± 0.71^{dC}	4.80 ± 0.27^{eB}
	12	12.50 ± 0.71^{dD}	5.09 ± 0.04^{dB}
	16	17.50 ± 0.71^{eE}	8.10 ± 0.07^{eC}
WW-TBZ	0	2.00 ± 0.0^{aA}	2.62 ± 0.36^{aA}
	4	8.25 ± 1.06^{eB}	3.06 ± 0.01^{dA}
	8	12.5 ± 0.71^{eC}	5.15 ± 0.03^{fB}
	12	17.5 ± 0.71^{eD}	6.09 ± 0.07^{eC}
	16	20.00 ± 0.0^{fE}	10.07 ± 0.1^{fD}
CH-OLE 1%	0	2.00 ± 0.0^{aA}	2.64 ± 0.39^{aA}
	4	5.25 ± 1.06^{dB}	2.95 ± 0.03^{cA}
	8	7.50 ± 0.71^{bC}	3.77 ± 0.16^{bB}
	12	7.50 ± 0.71^{bC}	4.44 ± 0.17^{bC}
	16	10.50 ± 0.71^{cD}	6.98 ± 0.05^{cD}
CH-OP 1%	0	2.00 ± 0.0^{aA}	2.64 ± 0.39^{aA}
	4	4.75 ± 0.35^{cB}	3.04 ± 0.01^{dA}
	8	8.75 ± 0.35^{cC}	4.05 ± 0.02^{dB}
	12	10.50 ± 0.71^{cD}	4.99 ± 0.03^{cC}
	16	13.50 ± 0.71^{dE}	7.15 ± 0.03^{dD}
CH-OLE 2%	0	2.00 ± 0.0^{aA}	2.64 ± 0.39^{aA}
	4	3.25 ± 1.06^{aB}	2.58 ± 0.07^{aA}
	8	6.25 ± 0.35^{aC}	3.57 ± 0.11^{aB}
	12	6.00 ± 1.41^{aC}	4.12 ± 0.07^{aC}
	16	8.00 ± 1.41^{aD}	5.94 ± 0.03^{aD}
CH-OPE 2%	0	2.00 ± 0.0^{aA}	2.64 ± 0.39^{aA}
	4	4.00 ± 1.41^{bB}	2.80 ± 0.16^{bA}
	8	6.00 ± 0.71^{aC}	3.94 ± 0.07^{cB}
	12	7.75 ± 0.35^{bD}	4.12 ± 0.07^{aC}
	16	9.00 ± 1.41^{bE}	6.05 ± 0.01^{bD}

$N = 3$.

a, b, c: Means with the same letter in the same column for each formula are not significantly different ($P > 0.05$).

A, B, C: Means with the same letter in the same column for each storage period are not significantly different ($P > 0.05$).

Conversely, the highest decreases in TPC were observed in uncoated fruits reached to 0.22 mg GAE/g at end of storage period. Obtained data noticed that CH-OOR may work as protective barrier on the fruit surface and reduce the oxygen supply. This finding was similar to reported results by Gol *et al.* (2013) and Wang and Gao (2013). Nevertheless, the decrease in TPC in longer storage period might be due to break down of cell structure released phenolics to be exposure to enzymatic oxidation (Macheix and Fleuriet 1990).

Total Flavonoids. The variation in TF content during cold storage of uncoated and coated fruits was presented in the same table. For the uncoated and coated fruits, TF content was progressively decreased during storage till the end of storage period recording greater decreases in uncoated fruits. Indeed, significant difference ($P < 0.05$) in TF content was noticed between all coated and uncoated fruits. However, no significant difference ($P > 0.05$) was found between both CH-OLE 1% and CH-OPE 1%, or between CH-OLE 2% and CH-OPE 2%. Expressively, the loss in TF in uncoated fruits was extremely rapid compared with coated fruits, namely the coated fruits with CH-OLE 2%. The lowest decrease in TF recorded 1.50 mg QE/100 g. These results are in agreement with Wang and Gao (2013) who suggested that the CH coating of strawberry fruits reducing the decreases in TF content.

Anthocyanin Contents. Surprisingly, during the preliminary stage of cold storage the uncoated as well as coated fruits showed a significant increase in anthocyanins as presented in Table 2. In contrast, the increase of anthocyanins in coated strawberry during this period was higher than uncoated strawberry. Gol *et al.* (2013) conveyed that during storage the fruits become redder and darker due to releasing the anthocyanins from the cell as a result of cell wall decomposition. After 12 days of storage, a gradual decline of anthocyanins was observed in strawberry fruits. A significant difference ($P < 0.05$) was observed between coated or uncoated fruits. The coated fruits with CH-OPE 2% recorded the highest anthocyanins content. Conversely, the uncoated fruits recorded the lowest anthocyanins to be 73.23 mg/100 g. While its recorded 44.81 mg/100 g. Indeed, edible coating can act as a gas barrier during cold storage to reduce the O_2 and CO_2 exchange. They also prevent the anthocyanins oxidation upon decomposition of cell wall. The presented results asserted that applying of CH-OOR films significantly reduced the anthocyanins deterioration as mentioned by Tzoumaki *et al.* (2009).

Ascorbic Acid. Data in Table 2, showed the effect of CH-OOR coating on AA content of strawberry fruits during cold storage. Obviously, the contents of AA in both coated and uncoated fruits gradually decreased with storage period elongation. Regardless coating treatment, AA was decreased 53.57 to 25.44 mg/100 g. Indeed, coating materials inhibited the deterioration of AA significantly. Likewise, coated strawberry with CH-OPE 2% caused delay in AA degradation rate. In addition, no significant difference ($P > 0.05$) was found between coated strawberry with CH-OLE1% and CH-OPE1%. However, the lowest decrease in AA was observed in coated fruits with CH-OLE 2% to be 35.45 mg/100 g at end of storage period.

FIG. 1. EFFECT OF CH AND CH-OOR WITH DIFFERENT CONCENTRATION ON *R. STOLONIFER* GROWTH IN STRAWBERRY FRUITS DURING STORAGE AT 4 ± 1C

The CH incorporated films may reduce O_2 diffusion, slow down the ripening rate, consequently better preserve AA and may delay senescence of fruits (Xing *et al.* 2011). Similar results have been mentioned that CH-films retarded the decrease of AA in strawberry fruits (Gol *et al.* 2013; Wang and Gao 2013).

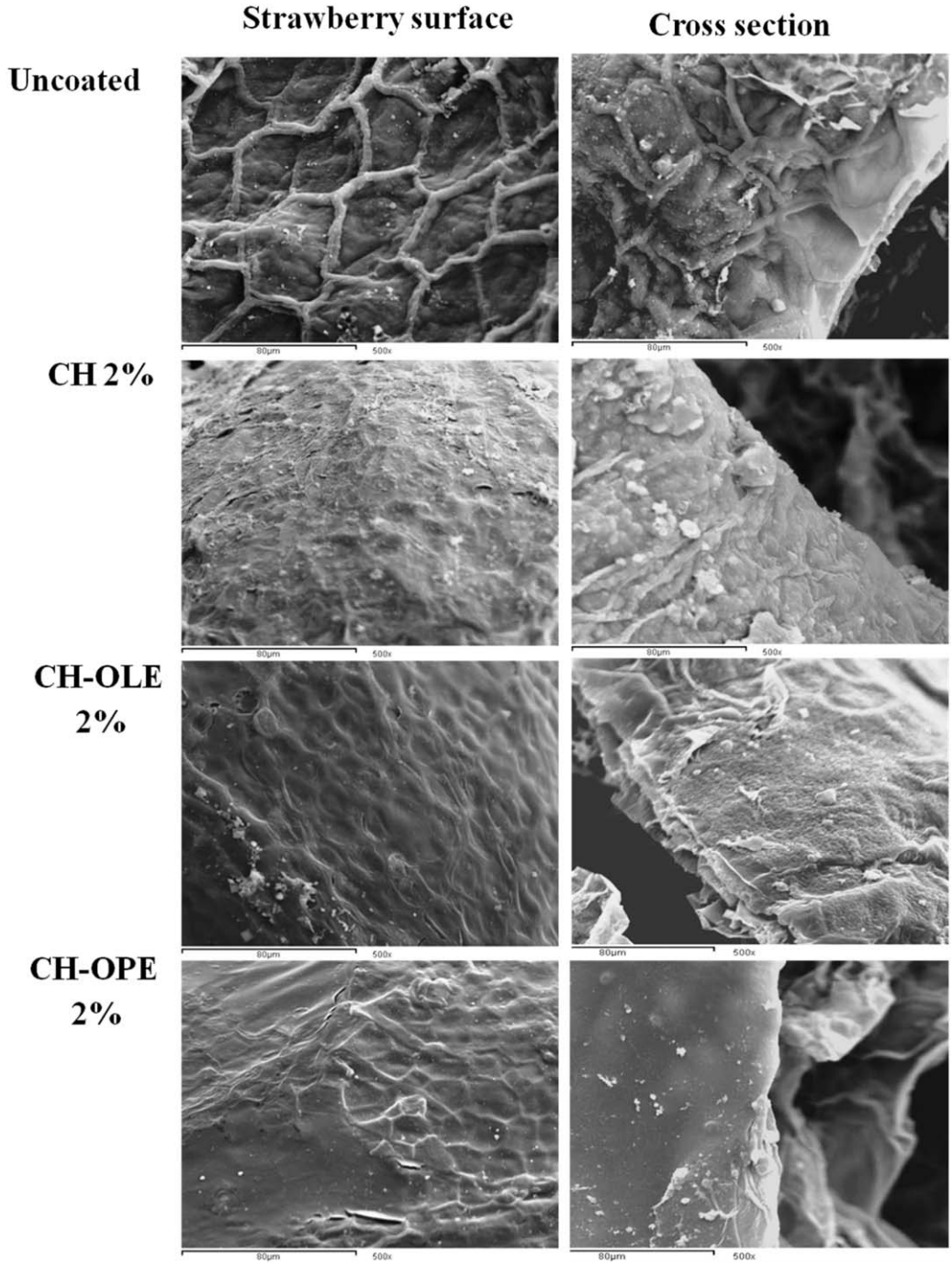

FIG. 2. SCANNING ELECTRON MICROGRAPHS OF SURFACE AND CROSS-SECTION OF COATED APPLE FRUITS WITH DIFFERENT FORMULAS OF CH-OOR CHITOSAN (CH) AND INCROPORATED CHITOSAN WITH OLIVE OIL RESIDUES (CH-OOR) FILMS

Antioxidant Activity. The stability of AOA in uncoated and coated strawberry fruits during cold storage was studied and showed in Table 2. Similarly, during the storage period the AOA decreased, especially uncoated fruits were decreased rapidly compared with coated fruits. Arguably, the AOA decreased significantly ($P < 0.05$) from 16.69 to 6.80 µmol TE/g in uncoated strawberry after 8 days. In contrast, coated fruits recorded slow decreases in AOA during cold storage. However, low decremental rate had been observed in coated fruits. Coating of strawberry fruits with CH-OLE 2% or CH-OPE 2% exhibit the lowest decreases rather than WW-TBZ 0.1%. No significant difference ($P > 0.05$) was found

among CH, CH-OPE 1% and CH-OLE 1%. A few studied were found about the effect of edible films coating prevented the loss of AOA in strawberry (Wang and Gao 2013).

Microbiological Examination of Infected Strawberry Fruits during Cold Storage

Generally, the infected area was increased with increasing the storage periods from 2 to 15 mm as illustrated in Table 3. Indeed, the decayed area of fruits reduced significantly compared with uncoated fruits. Consequently, the fruits were coated with CH-OLE 2% recorded the highest fruit in decrease of decayed area compared with both CH and WW-TBZ coated and uncoated fruits. Obviously, the highest observed area was 26.50 mm in uncoated strawberry at the end of storage. While, lowest observed area was 8.00 mm in strawberry with CH-OLE 2%. The CH and CH-OOR were more effective than commercial coating material (WW-TBZ) on growth of fungal strains as portrayed in Fig. 1. These motivated results could encourage the food handlers to replace the chemical coating materials with presented coatings formulas in this study. El-Ghaouth *et al.* (1991) suggested that CH induces chitinase, as defense enzyme catalyzes the hydrolysis of chitin, preventing or delayed fungi growth on fruit surface. Also, these results are complimentary to those of Park *et al.* (2005).

The fungal count of *R. stolonifer* as logarithmic number (log cfu/g) was presented in Table 3. The fungal count of *R. stolonifer* was increased rabidly during the cold storage in uncoated fruits. The lowest fungal count was recorded with coated fruits with CH-OLE 2% or CH-OPE 2%. It's bearing in mind that the growth of fungi strains on the surface or the cross section of the fruit being one of the causes of corruption for these fruits and malformed appearance. It leads to increase the weight loss for these fruits (see obtained weight loss analysis results). CH-OOR films were more effective in controlling of fungal growth during cold storage compared with either commercial waxing or CH only as shown in Table 3. These finding are confirmed by Rodríguez *et al.* (2003) and Park *et al.* (2005) who reported that CH coating delayed the growth of *Rhizopus* sp. and *Cladosporium* sp. in strawberry and pizza.

Microstructure of Strawberry Fruits

In order to study the homogeneity of CH or CH-OOR coating films on strawberry surface, the micrographs and cross-section of both uncoated and coated strawberry fruits were photographed using SEM and accessible in Fig. 2. It was observed that strawberry coated with CH or CH-OOR showed uniform coating distribution, since it was impossible to see any noncoated cellular structure, while pores weren't observed in these coated samples. The higher percentage of covered surface relates to the higher water vapor resistance which slowed respiration process and water loss as observed in coated strawberry with CH-OLE 2% and CH-OPE 2%. Also, The CH coating made the surface of the material was covering all irregularities in the fruits skin. This indicated that the extensibility of the liquid dispersion on the covered fruit surface plays an important role in limiting water migration from the samples (Villalobos-Carvajal *et al.* 2009).

CONCLUSIONS

The results of the present study asserted that the coatings by CH or CH-OOR have a beneficial impact on the quality retention of cold stored strawberry fruits. The coatings by CH-OOR were the most effective approach for quality preservation of strawberry fruits especially CH-OLE 2%. Regarding it resulted in a significant delay in weight loss, TSS, TS, TA, AA, anthocyanins and had positive effects on maintaining higher concentrations of TPC, TF and their AOA. The use of OOR also maintained lower activities of cell wall deterioration as calculated by MDA. Likewise, the incorporation of OOR to CH enhanced it antifungal activity as shown against *R. stolonifer* counted and decay area. Hence, coatings of strawberry fruits by CH-OOR may be useful for improving the postharvest quality and shelf-life comparing with CH only or WW-TBZ.

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