



Enhancing the keeping quality of fresh strawberry using chitosan-incorporated olive processing wastes

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2

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Thiabendazole (PubChem CID: 5430).

ABSTRACT

Not only, strawberry fruits have a very short shelf life, but also their bioactive substances were declined during postharvest. This study is aimed at determining the efficacy of edible coating enrichment with olive wastes polyphenols based chitosan on components of cold-stored strawberry fruits. Fruits were sprayed with five different coating formulas compared to water wax incorporated with thiabendazole (WW-TBZ) and uncoated fruits served as control. Then, some freshness parameters, decay area and microstructure observation were assayed. Indeed, the losses of each parameter in uncoated fruits were extremely rapid compared with coated fruits. Conversely, malondialdehyde and decay area significantly increased in uncoated fruits compared with coated fruits. Amazingly, the addendum of olive leaves extract into chitosan coating was expressively reduced the gradual decline in total phenolics, flavonoids, antioxidants, ascorbic acid and malondialdehyde. Whereas, olive pomace extract recorded the lowest influencing on anthocyanins during storage at 4 ± 1 °C for 16 day. In addition, both olive wastes extracts significantly enhanced the bioactive substances compared with WW-TBZ. Then, fruits coated with chitosan incorporation coating solution showed uniform coating distribution and no pores were found. Thus, olive wastes extracts integration into chitosan based coating led to keep the bioactive substances of cold-stored strawberry fruits.

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1. Introduction

Egypt is globally ranked as the first olive production in quantity per hectare⁻¹ to be 9.788 kg ha⁻¹ (FAO, 2013a). After olive oil processing, olive oil wastes (OOW) are discarded either olive leaves 10% (Bouaziz, Fki, Jemai, Ayadi & Sayadi, 2008) or olive pomace ≤ 70% (Sánchez & Ruiz, 2006). It causes economic losses and some ecological problems. Furthermore, it considers as promising sources for polyphenols, flavonoids and other bioactive constituents (Apostolakis, Grigorakis & Makris, 2014; Eid et al.; Guinda et al.; Pains et al., 2015; Talhaoui et al., 2014). Thus, OOW is revalorized before with polyesters and polylactic films (Marcos et al., 2014; Özge, Çam & Turhan, 2013). Afterwards, Egypt is contributed by 5.5% in the global production of strawberry around

242.29 tones, occupies the fifth production country world widely (FAO, 2013b). It is unique, highly desirable aroma and phytochemicals (Gasperotti et al.; Sun, Liu, Yang, Slovin & Chen, 2014; Van De velde et al., 2013). On the other hand, these components are dramatically decreased during postharvest (Ayala-Zavala, Wang, Wang & González-Aguilar, 2004; Carbone, Giannini, Picchi, Lo Scalzo & Cecchini, 2011; Fawbush, Nock & Watkins, 2009; Supapvanich, Pimsaga & Srisujan, 2011). Moreover, the pathogenic microorganisms may be growth in fruit's surface during postharvest. It can be promote decay, produce mycotoxins and degrade bioactive substances (Matthes & Schmitz-Eiberger, 2012). Commonly, these challenges might be fixed using coating treatments like commercial waxes such as (WW-TBZ). But, it causes some dangerous side effects (List, 2005). Therefore, modern trends using some natural polymers such as chitosan (CH) incorporated with natural additives like food processing wastes was recently discussed in fruits coating (Perdones, Vargas, Atarés & Chiralt, 2014; Shao et al., 2015; G. Yang et al., 2014). The CH (poly B-(1,4) N-acetyl-D-glucosamine) is the second most abundant polysaccharide found in nature after cellulose (Martínez-Camacho

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et al., 2010). It has good filmogenic, antimicrobial activity, GRAS and environmental friendly (Aider, 2010; Fernandez-Saiz, Lagaron & Ocio, 2009; Kean & Thanou, 2010; Ojagh, Rezaei, Razavi & Hosseini, 2010). However, to our knowledge, there is no scientific literature available regarding the effect of OOW incorporation with CH coating solution on the bioactive substances of strawberry fruits during postharvest. Therefore, the present study has been undertaken with the objective of elucidating the potential of CH only or combinations with both OOW on shelf-life extension and bioactive substances keeping of cold-stored strawberry fruits comparing with commercial waxing comparable WW-TBZ.

2. Materials and methods

2.1. Reagents and Solutions

1, 1-diphenyl-2 picrylhydrazyl radical (DPPH[•]), 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, Co., Germany. Chitosan (> 90% deacetylation, high molecular weight and viscosity 500–2000 cps) was gotten from Oxford Co., India. 2, 6-dichlorophenol-indophenol and Folin-Ciocalteu reagent were obtained from Fluka Biochemical, Co., Switzerland. Gallic acid Serva, Biochemical, Co., New York. Thiabendazole and water wax[®], Fomesa Fruitech, Co., Spain.

2.2. Microbial strain and media

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

2.3. Raw materials

- a. Olive (*Olea. europaea* var. *Kronakii*) wastes including olive leaves and pomace were obtained from Cairo for oil industry, Co., industrial zone, 6th October City, Egypt.
- b. Fresh strawberry fruits (*Fragaria ananassa* var. *Festival*) were obtained from Abo-Rahia farm, Toukh city, Egypt.

2.4. Methods

2.4.1. Olive oil processing wastes preparation and extraction

Both wastes were oven dried (Tit Axon S.R.L via Canova, Italy) at 40–50 °C gradually for 12 h. Subsequently, these were milled by grinder (Severin, type 3871, Germany) and passed through a 60 mesh sieve to obtain a fine homogenous powder. They were packed directly in dark glass jars then kept at -18 ± 1 °C until use. On the other hand, both olive leaves and olive pomace were individually mixed with ethanol 80% as (1:20, w/v) in dark bottles with shaking at 120 rpm for 24 h. The mixtures 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 40 °C according to Lafka, Lazou, Si-nanoglou, and Lazos (2011).

2.4.2. Film forming solution

The incorporated CH solutions with ethanolic olive leaf extracts and olive pomace extracts were prepared according to Gol, Patel, and Rao (2013) with some modifications. A 20 g L⁻¹ CH was dispersed in an aqueous solution of glacial acetic acid (0.5%, v/v) at 40 °C. The solution was heated and agitated constantly for 12 h

then 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. Both OOW extracts 1 and 2% (v/v) were added and mixed to achieve complete dispersion.

2.4.3. Strawberry fruits coating treatments

Strawberry fruits were sorted for uniform size, color, maturity and for being free of visible defect as well as decay. Then, they were sanitized by sodium hypochlorite solution 250 mg L⁻¹ and washed with distilled water to eliminate chlorine traces. Subsequently, cross-shaped wounds were made on the strawberry using sterilized puncher and inoculated by *R. stolonifer* spores suspension (10⁵ spores mL⁻¹). The coating solutions (as described above Section 2.4.2) were sprayed on the whole fruits surface using a Multi-function hand 2 L pressure sprayer (Ningbo Synkemi. Co., type SK-2B, China) twice and air-dried at ambient temperature for 2 h. Seven groups of strawberry were prepared in total uncoated (control), CH (2% w/v), Chitosan-Olive leaves extracts CH-OLE (1 and 2% w/v), Chitosan-Olive pomace extracts CH-OPE (1 and 2% w/v), WW-TBZ 0.1% as positive control according to Zhang and Quantick (1997). The Fruits were packed in boxes (~3 fruit per box) and wrap with polyethylene sheets, then stored at 4 ± 1 °C for 16 day. The bioactive substances and decay area of uncoated and coated fruits were evaluated at the beginning of the experiment (i.e. 0 days) and after 4, 8, 12 and 16 day.

2.4.4. Bioactive substances of coated strawberry fruits

2.4.4.1. Anthocyanins contents. The Anthocyanins content of strawberry fruits were determined according to Fuleki and Francis (1968). A 5 g strawberry samples were extracted with 45 mL of acidified ethanol (95% ethanol: HCl 1.5N 85:15) for 2 h at room temperature in the dark, filtered and measured at 535 nm. The data were calculated based fresh weight (fw) in all next parameters.

2.4.4.2. Ascorbic acid. The ascorbic acid content in different strawberry fruits during storage periods were determined using 2, 6-dichlorophenol-indophenol titrimetric method according to Thimmaiah (1999). A pure ascorbic acid was used to prepare a standard solution (1 mg mL⁻¹).

2.4.4.3. Total phenolics contents. The total phenolics compounds (TPC) for acetone extracts of strawberry were determined according to (Pineli et al., 2011). In brief, 200 µL of each sample was mixed with 1 mL of 10-fold diluted Folin-Ciocalteu reagent. The reaction was stopped after 5 min by 1 mL of 75 g L⁻¹ Na₂CO₃ then 1.5 mL distilled water was added. The mixtures were incubated in dark for 60 min then the absorbance at 760 nm was measured. The TPC was expressed as gallic acid equivalents (mg of GAE 100 g⁻¹dw) using the following equation based on the calibration curve:

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

where Y is the concentration and x is the absorbance.

2.4.4.4. Total flavonoids. The total flavonoids content (TF) for acetone extracts of strawberry was determined according to Mohdaly, Hassanien, Mahmoud, Sarhan, and Smetanska (2012). A 0.5 mL aliquot of 20 g L⁻¹ AlCl₃ ethanolic solution was added to 0.5 mL of extracts and mixed well. Then it was kept for 1 h at room temperature and the absorbance at 420 nm was measured. The final concentration of TF was expressed as quercetin equivalent (mg QEg⁻¹dw) which was calculated using the following equation based on the calibration curve:

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

where Y is the concentration and x is the absorbance.

2.4.4.5. Antioxidant activity. The antioxidant activity (AOA) of strawberry acetone extracts were evaluated according to (Pineli et al., 2011). A 0.1 mL extract was added to 3.9 mL of DPPH[•] methanolic solution ($0.0025 \text{ g } 100 \text{ mL}^{-1}$). After the solution had been allowed to stand in the dark for 60 min, the absorbance at 517 nm was measured. The final results were expressed as Trolox equivalents ($\mu\text{mol TE g}^{-1}\text{dw}$).

2.4.4.6. Determination of fruits tissue deterioration. The strawberry pulp (2.0 g) was homogenized in 6 mL of 10% trichloroacetic acid then centrifuged for 15 min at 6000 $\times g$. A 2 mL from supernatant was mixed with 6 mL of 0.6% thiobarbituric acid then heated to 100 °C for 20 min, quickly cooled and centrifuged at 6000 $\times g$ for 10 min. The supernatant was collected and absorbance was measured at 450, 532 and 600 nm. The Malondialdehyde concentration was calculated according to Hong, Xie, Zhang, Sun, and Gong (2012) using the following equation:

$$\text{Malondialdehyde} = \left[(6.45 \times (A_{532} - A_{600})) - (0.56 \times A_{450}) \right] \quad (3)$$

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.

2.4.5. Determination of decay area

The strawberry fruits were inoculated by *R. stolonifer* spores (as described above Section 2.4.3), then they were coated with different coating formulas. After that, the 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).

2.4.6. Microstructure analysis

The strawberry skins' surface and cross-section microstructure which coated by CH (2%), CH-OLE (2%), CH-OPE (2%), and uncoated fruits were examined under scanning electron microscope. Tissues from different treatments were fixed in 4% glutaraldehyde in 0.2 M sodium cacodylate buffer (pH 4.1) for 4 h, then fixated later in osmium tetroxide for 2 h. Fixed tissues were rinsed in the 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 copper 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 scanning electron microscope (JXA-840A, Electron Probe Micro analyzer-JEOL, Japan).

2.4.7. Statistical analysis

The statistical analysis was carried out using SPSS program (ver. 19) with multi-function utility regarding to the experimental design and multiple comparisons were carried out applying LSD according to Steel, Torrie, and Dickey (1997).

3. Results and discussions

3.1. Anthocyanins content

Surprisingly, during the preliminary stage of cold storage the uncoated and coated fruits showed a significant increase in anthocyanins (Fig. 1). Gol et al. (2013) reported that the fruits become darker during storage due to releasing anthocyanins from

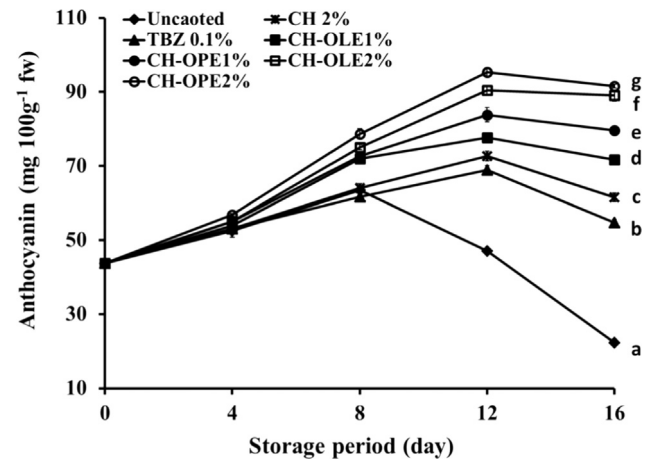


Fig. 1. Monitoring of anthocyanins for coated and uncoated strawberry fruits with different chitosan based coating formulas during cold storage at 4 ± 1 °C, (Mean \pm SD, $n=3$).

the cell after it decomposition. After 12 day of storage, a gradual decline of anthocyanins was observed in strawberry fruits. Significant difference ($P < 0.05$) was investigated among treatments. Nevertheless, coated strawberry with CH-OPE 2% recorded the highest anthocyanins content at the end of storage. The presented results asserted that applying of CH-OOW meaningfully reduced the decreases in anthocyanins compared with WW-TBZ. This may be their filmogenic property that prevents the anthocyanins oxidation upon decomposition of cell wall as declared (Tzoumaki, Biliaderis & Vasilakakis, 2009; Yang et al., 2014).

3.2. Ascorbic acid

Data in Fig. 2 displayed the effect of different coating based CH-OOW on ascorbic acid contents of cold storage strawberry fruits. Obviously, the contents of ascorbic acid gradually declined in both coated and uncoated fruits with postharvest elongation. Indeed, coating materials expressively inhibited ascorbic acid deterioration. In addition, no significant difference ($p > 0.05$) was found between coated strawberry with CH-OLE1% and CH-OPE1%. However, the lowest decreases in ascorbic acid were detected in coated fruits with CH-OLE 2% around $35.45 \text{ mg } 100\text{g}^{-1} \text{ fw}$ at the end of storage period. The CH-OOW films may retard ascorbic acid in strawberry fruits (Gol et al., 2013; Wang & Gao, 2013).

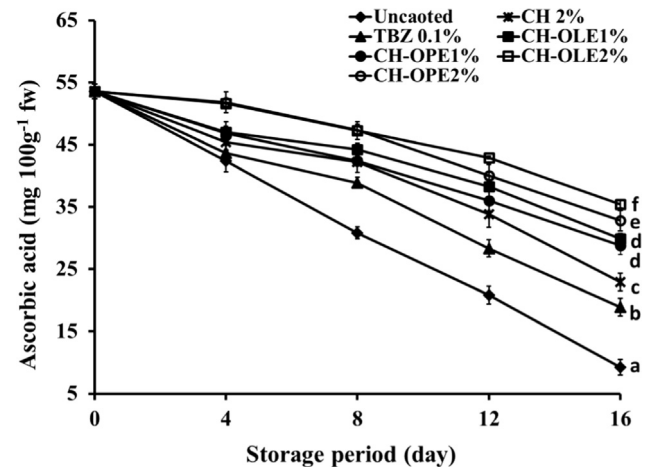


Fig. 2. Changing of ascorbic acid in coated and uncoated strawberry fruits with different chitosan based coating formulas during cold storage at 4 ± 1 °C, (Mean \pm SD, $n=3$).

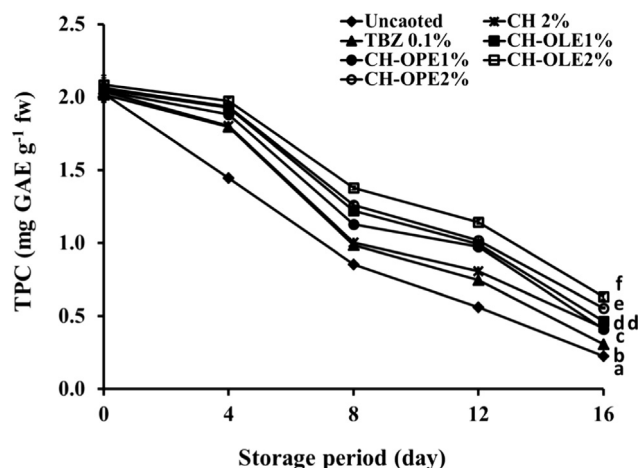


Fig. 3. Monitoring of TPC for coated and uncoated strawberry fruits with different chitosan based coating formulas during cold storage at 4 ± 1 °C, (Mean \pm SD, $n=3$).

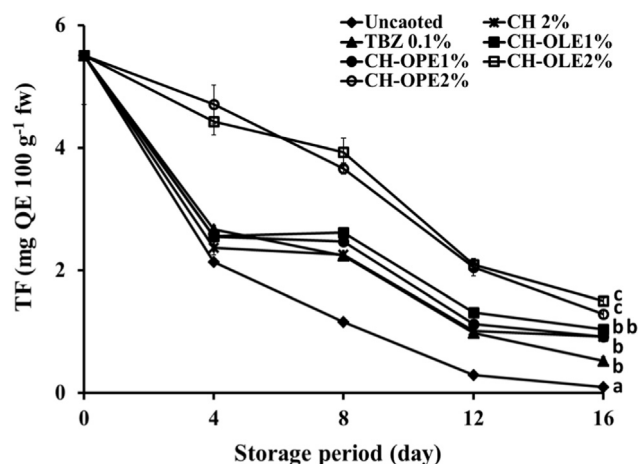


Fig. 4. Monitoring of TF for coated and uncoated strawberry fruits with different chitosan based coating formulas during cold storage at 4 ± 1 °C, (Mean \pm SD, $n=3$).

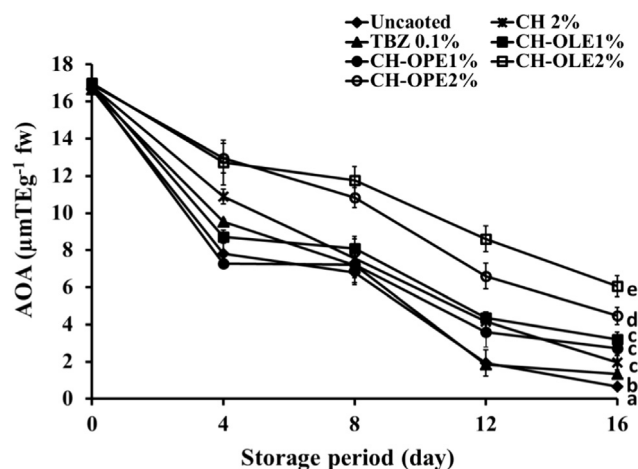


Fig. 5. Monitoring of AOA for coated and uncoated strawberry fruits with different chitosan based coating formulas during cold storage at 4 ± 1 °C, (Mean \pm SD, $n=3$).

3.3. Total phenolics compounds

In all coated fruits significantly ($p < 0.05$) drops in the TPC degradation compared with uncoated fruits as offered in Fig. 3. And this matter due to breakdown of cell structure released phenolics to be exposure to enzymatic oxidation (Macheix & Fleuriot,

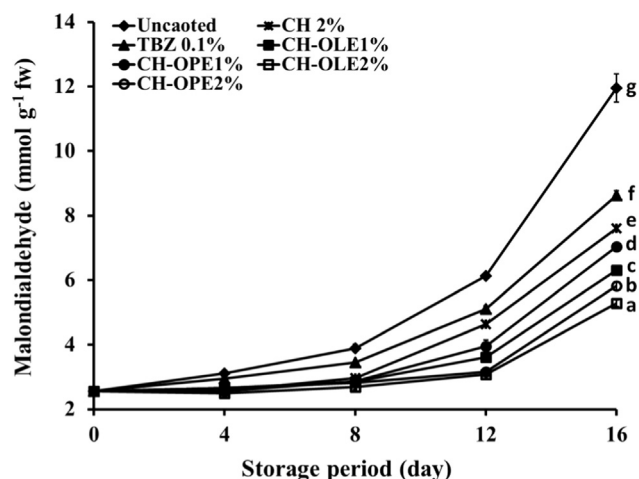


Fig. 6. Effect of CH, CH-OOW and WW-TBZ coating solution on malondialdehyde in strawberry fruits during cold storage at 4 ± 1 °C, (Mean \pm SD, $n=3$).

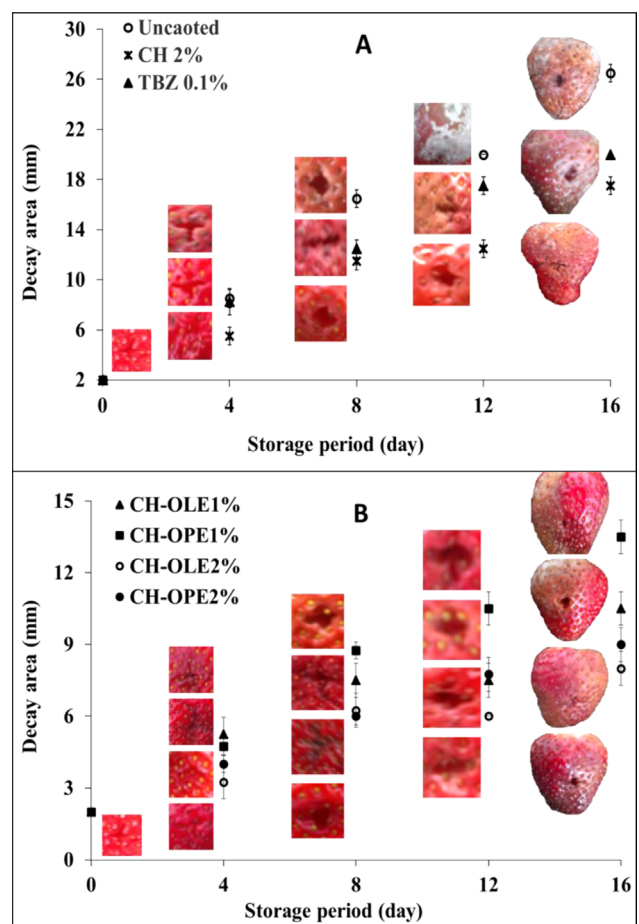


Fig. 7. Decay area in uncoated strawberry and coated with CH and WW-TBZ (A), CH-OOW (B) infected with *R. stolonifer* during cold storage at 4 ± 1 °C, (Mean \pm SD, $n=3$).

1990). Obviously, the lowest falls in TPC showed in coated strawberry with CH-OLE 2% nearby $0.63 \text{ mg GAE g}^{-1} \text{ fw}$. Vice versa, the highest reductions in TPC detected in uncoated fruits being $0.22 \text{ mg GAE g}^{-1} \text{ fw}$ at the end of storage period. It was preceded by WW-TBZ to reach $0.30 \text{ mg GAE g}^{-1} \text{ fw}$. Obtained data noticed that CH-OOW might work as protective barrier on the fruits' surface and reduce the oxygen supply. This finding was similar Gol et al. (2013), Wang and Gao (2013).

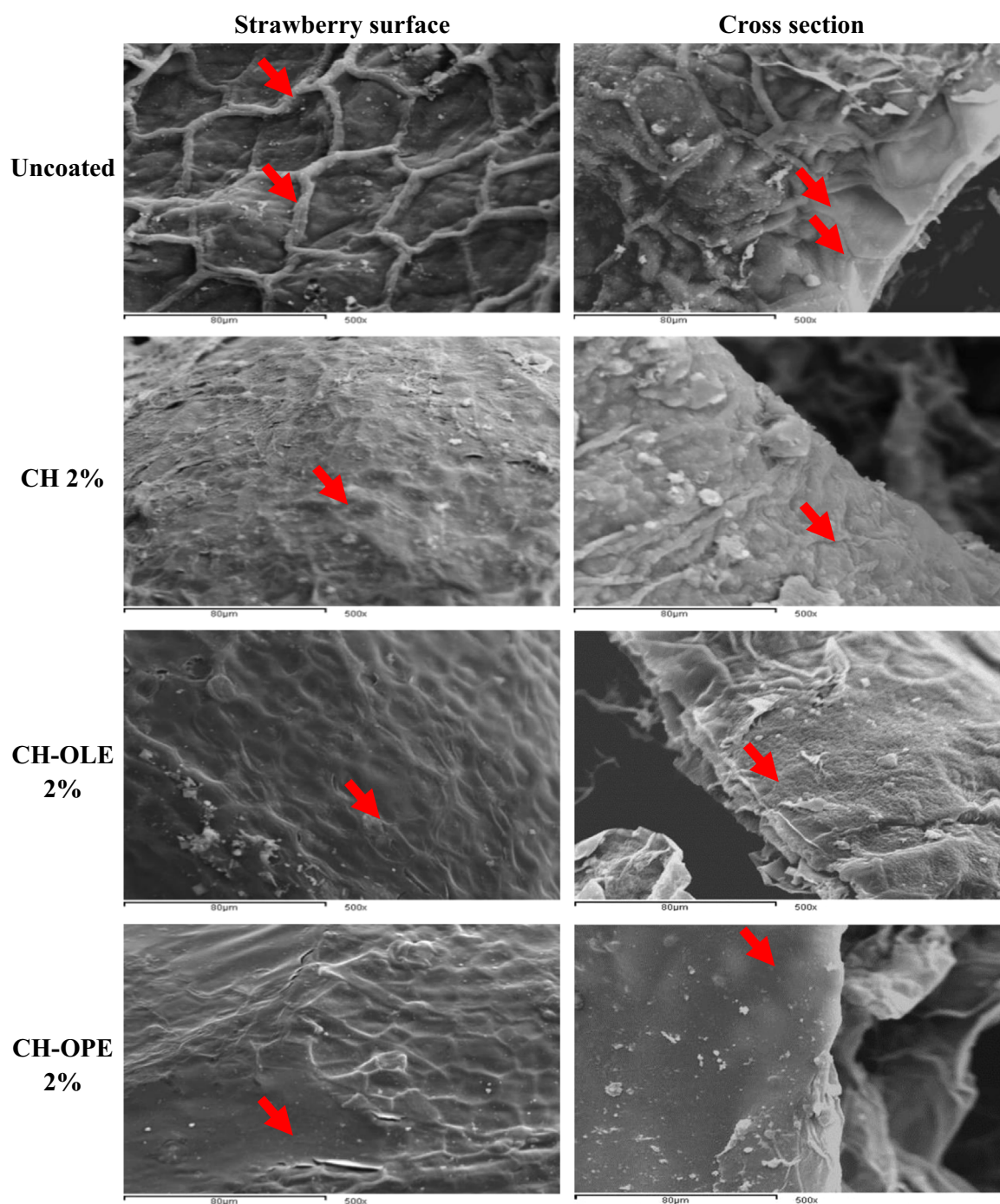


Fig. 8. Scanning electron micrographs of surface and cross-section of uncoated and coated strawberry fruits with CH, CH-OLE 2% and CH-OPE 2% formulas, ($n=1$).

3.4. Total flavonoids

The variation in TF contents during cold storage of uncoated and coated fruits was exhibited in Fig. 4. In both fruits, TF was progressively declined during storage period recording greater decreases in uncoated fruits. Surely, no significant difference ($p > 0.05$) was found among CH, WW-TBZ, CH-OLE 1% and CH-OPE 1% or between CH-OLE 2% and CH-OPE 2%. However, the coating with CH-OLE 2% was reduced the gradual deterioration in TF to be $1.49 \text{ mg QE } 100 \text{ g}^{-1} \text{ fw}$. Wang and Gao (2013) suggested that the CH coating of strawberry fruits reducing the decreases in TF content.

3.5. Antioxidant activity

The stability of AOA in uncoated and coated strawberry fruits during cold storage was studied then data are shown in Fig. 5. Similarly, during the storage period the AOA decreased especially in uncoated fruits compared with coated fruits. Arguably, the AOA significantly reduced ($P < 0.05$) from 16.69 to $6.80 \text{ } \mu\text{mol TE g}^{-1} \text{ fw}$ in uncoated strawberry after 8 day. However, low decremental rate had been witnessed 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 established between coated strawberry by

CH and CH-OPE 1% or CH-OLE 1%. Few studies were found about the effect of edible films coating prevented the loss of AOA in strawberry (Wang & Gao, 2013).

3.6. Malondialdehyde contents

The malondialdehyde contents have been used as direct indicator of cell membrane injury and index of cell oxidative damage (Xu et al., 2009). As revealed in Fig. 6, malondialdehyde contents in strawberry pulp amplified during cold storage period from 2.55 and 7.51 mmol g⁻¹ fw. Obviously, the uncoated fruits had high significantly malondialdehyde than coated fruits. To enumerate that the malondialdehyde in uncoated strawberry was 5.52 mmol g⁻¹ fw, however it was reached in strawberry coated with CH-OLE 2% to 3.22 mmol g⁻¹ fw. Generally, strawberry was coated with CH-OLE 2% noted the lowest incremental rate of malondialdehyde at different storage periods. The dramatically increases of malondialdehyde in uncoated strawberry was resulted soft and pale tissues. However, coating treatment was improved the membrane integrity and increased the keeping quality. There are no studies was found about this issues.

3.7. Decay area

Generally, the infected area was gradually increased with increasing the storage periods as shown in Fig. 7. Indeed, the decayed area of coated strawberry fruits was smaller reduced significantly compared with the uncoated fruits. Consequently, the fruits that were coated with CH-OLE 2% recorded the highest decrease fruit in decay area compared with other formulas. The mean value of the decay area in the uncoated and CH-OLE 2% were 14.70 and 5.10 mm. Obviously, the highest detected area was 26.50 mm in uncoated strawberry at the end of storage. Alternatively, the lowest observed area was 8.00 mm in coated strawberry with CH-OLE 2%. The CH and CH-OOW were more effective than the commercial coating material of WW-TBZ on fungal strain growth as publicized in Fig. 7. These motivated results could encourage the food handlers to replace the chemical coating materials with the presented coatings formulas of the current study. These findings are confirmed by (Park, Stan, Daeschel & Zhao, 2005; Rodríguez, Ramos & Agulló, 2003), who reported that CH coating delayed the growth of *Rhizopus* sp. and *Cladosporium* sp. in strawberry and pizza.

3.8. Microstructure observation

In order to study the homogeneity of CH or CH-OOW coating on strawberry fruits skins, the micrographs of the surface and cross-section were photographed using scanning electron microscope and presented in Fig. 8. It was observed that the coated strawberry especially with CH-OOW showed uniform coating distribution and pores were not seen in these coated samples. The higher percentage of covered surface relates to the advanced water vapor resistance that slowed respiration process and water loss as observed in coated strawberry with CH-OLE 2% and CH-OPE 2%. Therefore, the coated strawberry surface with CH or CH-OOW was shiny, while they coated with CH-OOW coating were more mats. Additionally, the CH and CH-OOW 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, Hernández-Muñoz, Albors & Chiralt, 2009).

4. Conclusion

The results of the present study asserted that the incorporated of olive oil processing wastes into CH was improved the bioactive substances and freshness quality for cold-stored strawberry fruits. In addition, the coatings of CH or CH-OOW have a beneficial impact on the quality retention of cold storage strawberry fruits especially CH-OLE 2%. The use of olive oil processing wastes correspondingly maintained lower activities of cell wall deterioration as calculated by malondialdehyde and decay area. Likewise, CH-OOW was resulted in a significant delay in anthocyanins, total phenolics, flavonoids and antioxidants. Also, CH-OOW was fully covered the whole surface of strawberry fruits in term of seen any pores. Hence, coatings of strawberry fruit with CH-OOW may be useful for improving postharvest quality and shelf-life comparing to CH only or WW-TBZ.

Conflict of interest

Author has declared that there is no conflict of interest.

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