

# Improving the shelf-life stability of apple and strawberry fruits applying chitosan-incorporated olive oil processing residues coating



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## ABSTRACT

Recently, antioxidants and antimicrobials incorporation into edible films are one of the novel techniques in food processing. This study is aimed at determining the applicability of olive oil residues extracts (OOR) incorporated with chitosan (CH) films for improving the shelf-life stability of apple and strawberry fruits. After OOR incorporating, antimicrobial and physical parameters of each formula were investigated. Then, the keeping quality parameters of infected cold stored apple and strawberry were assayed. Indeed, addition of OOR to CH led to increase its antifungal and antibacterial activities against the tested strains. Regardless, the inhibition percentage was clearly high against *Penicillium expansum* compared with *Rhizopus stolonifer* *in vitro* and *in vivo*. Moreover, 20 g kg<sup>-1</sup> OOR was slightly affected the film appearance; but significantly influenced the thickness and solubility. Amazingly, the CH-OOR was reduced significantly the gradual decline both coated fruits in their microbiological features. Therefore, integration of OOR into CH can be used to improve the inhibition properties of CH based film against spoilage and pathogenic strains. The olive leaves extract exhibited valuable efficiency than olive pomace extract in both *in vitro* and *in vivo*. Moreover, the result suggested that CH-OOR could be explored as a novel and potential natural edible coating to substitute the synthetic agents for apple and strawberry coating.

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## 1. Introduction

Not only food processing residues lead to economic losses, but also cause some environmental problems. However, they are promising sources of various bioactive substances (da Silva et al., 2014; Escobedo-Avellaneda, Gutiérrez-Urbe, Valdez-Fragoso, Torres, & Welti-Chanes, 2014; Moo-Huchin et al., 2015). Thus, they were revalorized before in edible films and coating solutions based chitosan (CH) to enhance the keeping and freshens quality of citrus (Shao et al., 2015), blueberries (Yang et al., 2014), strawberries (Khalifa, Barakat, El-Mansy, & Soliman, 2016; Perdones, Vargas, Atarés, & Chiralt, 2014; Yang et al., 2014), for instance. Contrariwise, Egypt is globally ranked as the first olive production in the quantity of the hectare<sup>-1</sup> to be 9.788 kg Ha<sup>-1</sup> (FAO, 2013). Surprisingly, olive oil residues (OOR) have contained

large amounts of bioactive substances (Brahmi, Mechri, Dhibi, & Hammami, 2013; Şahin & Şamlı, 2013), antioxidants (Brahmi, Mechri, Dabbou, Dhibi, & Hammami, 2012; Esteve, Marina, & García, 2015; Terpinč, Čeh, Ulrih, & Abramovič, 2012), antimicrobials (Keskin, Ceyhan, Uğur, & Dbeys, 2012). Accordingly, they were incorporated with polylactic acid and methylcellulose films (Ayana & Turhan, 2009; Özge, Çam, & Turhan, 2013).

CH (poly B-(1,4) N-acetyl-D-glucosamine) is linear polysaccharides that have many biological activities including antimicrobials (Aider, 2010; Ojagh, Rezaei, Razavi, & Hosseini, 2010), antioxidants (Siripatrawan & Harte, 2010), non-toxic (Ribeiro, Vicente, Teixeira, & Miranda, 2007), GRAS (Kean & Thanou, 2010) and good film-forming properties (Aider, 2010). Differentially, the pathogenic microorganisms grow on fruit's surface during postharvest which can promote decay, produce mycotoxins and degraded phytochemicals (Matthes & Schmitz-Eiberger, 2012). Commonly, these challenges might be fixed using coating by commercial wax like water wax (WW) incorporated with some additives such as thiabendazole (TBZ). The TBZ may cause some dangerous side effects (List, 2005). Therefore, modern trends using some natural polymers such as CH incorporated with natural additives was recently discussed. However, to our knowledge, there is no

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scientific literature available regarding the effect of CH-OOR on biological characteristics of apple and strawberry fruits. Accordingly, the present study aimed to examine the effect of OOR incorporated into CH film on its functional properties *in vivo* and *in vitro*. After the CH film being formed, the antimicrobial activity could be investigated. Keeping quality parameters were determined for coated apple and strawberry fruits.

## 2. Materials and methods

### 2.1. Reagents and microbial strains

Chitosan (>90% deacetylation, high molecular weight and viscosity 500–2000 cps) was gotten from Oxford Co., India. Thiabendazole and water wax<sup>®</sup> were obtained from Fomesa Fruitech, Spain. Sabouraud agar No. 402005 was obtained from Biolife Co., Italy. Mueller Hinton agar (MHA) No. SM 173, Mueller Hinton broth (MHB) and Potato dextrose broth (PDB) No. M 403 were obtained from Himedia Co., India. Also, *Penicillium expansum* ATCC 7861 and *Rhizopus stolonifer* ATCC 14037 were obtained from Cairo Microbiological Resource Center (MIRCEN), Fac. of Agric., Ain Shams Univ., Cairo, Egypt. As for the bacterial strains such as (*Bacillus cereus*, *Escherichia coli*, *E. coli* O16, *E. coli* O26, *E. coli* O103, *E. coli* O121, *E. coli* O157, *Listeria monocytogenes*, *Salmonella typhi*, *S. Typhimurium*, *Staphylococcus aureus* and *Yersinia Spp.*) were obtained from the Institute for Fermentation (Institut für Gärungsgewerbe, Berlin, Germany).

### 2.2. Raw materials

- Olive (*Olea europaea* var. *Kronakii*) residue leaves and olive pomace were obtained from Cairo for Oil Industry Co., industrial zone, 6th October city, Egypt.
- Fresh apple fruits (*Malus domestica* var. *Anna*) were obtained from an Alexandria Agriculture Farm, 70 km Cairo–Alexandria desert road, Egypt.
- Fresh strawberry fruits (*Fragaria ananassa* var. *Festival*) were obtained from Abo-Rahia farm, Toukh city, Egypt.

### 2.3. Analytical techniques

#### 2.3.1. Olive oil processing residues preparation and extraction

Both residues 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 fine homogenous powder. Afterword, they were packaged in dark glass jars then kept at  $-18 \pm 1$  °C until use. On the other hand, both olive leaves and pomace were individually mixed with ethanol 800 mL L<sup>-1</sup> 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. The filtrates were collected, then ethanol was removed by rotary evaporator (NE-1-Rikakikai Co., LTD, Japan) at 40 °C according to Lafka, Lazou, Sinanoglou, and Lazos (2011).

#### 2.3.2. Cultures propagation

Loop full from all bacterial cultures were inoculated into MHB then incubated at 37 °C for 12 h to make inoculation cultures. Oppositely, *P. expansum* and *R. stolonifer* spores were inoculated on sabouraud agar and incubated at 28 °C for 5 d. The fungal spore suspensions were prepared by washing the 5-old day's cultures by 10 mL tween 80 solution 1 mL L<sup>-1</sup> using glass rod to make a stock suspension solution and count using Thoma's cell with light microscopy.

#### 2.3.3. Film forming solution

The incorporated CH film with ethanolic olive leaf extracts (OLE) and olive pomace extracts (OPE) were prepared according to Gol, Patel, and Rao (2013) with some modifications. A 20 g L<sup>-1</sup> CH was dispersed in an aqueous solution of glacial acetic acid (5 mL L<sup>-1</sup>, v/v) at 40 °C. The solution was heated and agitated constantly for 12 h then pH was adjusted to 5.6 with 1 mol L<sup>-1</sup> NaOH. Subsequently, glycerol 16 mL L<sup>-1</sup> was added as a plasticizer (Sánchez-González et al., 2011). The OLE and OPE 10 and 20 g L<sup>-1</sup> (w/v) were added and mixed to achieve complete dispersion. Subsequently, they were degassed, left standing for 12 h at 25 °C and centrifuged at 8000g for 10 min. The solutions were then dispensed on polystyrene plates and left to dry for 24 h at 25 °C on a previously leveled surface until the total evaporation of the solvent. The dried films were peeled from the plate and maintained at 25 °C at a relative humidity of 45%. The physical properties of the different films based chitosan and the biological properties of different coating formulas based chitosan were aimed.

#### 2.3.4. Effect of olive oil residues combination with chitosan on its antimicrobial properties

**2.3.4.1. Antifungal properties.** The effect of OLE and OPE (10 and 20 g L<sup>-1</sup>) on antifungal properties of CH 20 g L<sup>-1</sup> edible coating solution was performed using mycelia yield assay according to Tripathi, Sharma, and Sharma (2009). PDB medium was prepared in 50 mL Erlenmeyer flasks and inoculated with 10<sup>5</sup> spore mL<sup>-1</sup> of *P. expansum* and *R. stolonifer*. The flasks were incubated at  $28 \pm 1$  °C with 120 rpm shaking. After 5 d, flasks containing mycelia were filtered through filter paper Whatman No.1 and washed. The mycelia were allowed to dry at 60 °C for 6 h and at 40 °C overnight. The mycelium dry weight was detected and growth inhibition percentage was calculated as:

$$\text{Growth inhibition \%} = [(DW_{\text{utf}} - DW_{\text{etf}}) / DW_{\text{utf}}] \times 100 \quad (1)$$

where: DW<sub>utf</sub>: dry weight of untreated fungal strain and DW<sub>etf</sub>: dry weight of treated fungal strain with CH solution and CH-OOR-incorporated coating solution.

**2.3.4.2. Antibacterial properties.** The screening of antibacterial activity of CH-OOR in different concentrations (5, 10, 20 and 30 g L<sup>-1</sup>) were performed using agar disc diffusion assay as described by Kotzekidou, Giannakidis, and Boulamatsis (2008). The bacterial strains (*B. cereus*, *E. coli*, *E. coli* O16, *E. coli* O26, *E. coli* O103, *E. coli* O121, *E. coli* O157, *L. monocytogenes*, *S. typhi*, *S. Typhimurium*, *Staph. aureus* and *Yersinia Spp.*) were propagated by adding a loop full from each strain into MHB then incubated at 37 °C for 12 h. Appropriate volume from each culture was mixed with sterilized MHA to set an inoculums as  $\sim 10^{-6}$  cell mL<sup>-1</sup> then poured in sterilized petri dishes. Consequently, CH-OOR was sterilized by 0.45 μm filters (Minisart<sup>®</sup>, Germany). Sterile filter paper discs 6 mm were immersed into sterilized solutions for 5 s then put immediately onto the surface of the solid cultures. The plates were incubated at 37 °C for 24–48 h. After incubation period, the inhibition zones around discs were measured. The photos were captured by an Olympus digital camera 8 MP model FS-32.

#### 2.3.5. Characterization of different CH-OOR films

**2.3.5.1. Film thickness.** After film formation, the thickness of each film using a micrometer was measured. Three measurements were performed at various points on each film according to (Sánchez-González, González-Martínez, Chiralt, & Cháfer, 2010).

**2.3.5.2. Film solubility.** The initial dry matter of the films was determined by drying 0.02 m diameter disks in oven, at 100 °C for 24 h. Other disks were cut, weighed and immersed in 50 mL of distilled water, with periodic stirring, for 24 h at 25 °C. Then, the films were taken out and dried (100 °C for 24 h) to determine the final dry matter. The solubility was reported as the difference between the initial and final dry matter weight according to Dutta, Tripathi, Mehrotra, and Dutta (2009).

### 2.3.6. Determination of the microbiological and freshness quality attributes of coated fruits

**2.3.6.1. Effect of olive residues extracts incorporated with chitosan on the growth of *P. expansum* and *R. stolonifer* fungal strains.** The effect of CH, CH-OOR-incorporated edible solution (as described above Section 2.3.3) and WW-TBZ were evaluated *in vivo* against *P. expansum* *R. stolonifer* according to Maqbool, Ali, Ramachandran, Smith, and Alderson (2010) with slight modifications. The apple and strawberry fruits were disinfected with sodium hypochlorite (250 mg L<sup>-1</sup>) for 2 min, then dipped in sterilized water for 2 min and left at ambient temperature until they dried completely. No residual of chlorine was detected upon applying a chlorine test according to A.O.A.C. (2000). Subsequently, cross-shaped wounds were made on the apple and strawberry using sterilized puncher and inoculated by *P. expansum* and *R. stolonifer* spores suspension (10<sup>5</sup> spores mL<sup>-1</sup>), respectively. After 15 min, the inoculated fruits were sprayed by the prepared coating solutions using a Multi-function hand 2L pressure sprayer (Ningbo Synkemi. Co., type SK-2B, China) twice and air-dried at ambient temperature for 2 h. Seven groups of samples were prepared in total uncoated (control), coated with CH (20 g L<sup>-1</sup> w/v), coated with CH-OLE (10 g L<sup>-1</sup> w/v), coated with CH-OLE (20 g L<sup>-1</sup> w/v), coated with CH-OPE (10 g L<sup>-1</sup> w/v), coated with CH-OPE (20 g L<sup>-1</sup> w/v), coated with WW-TBZ 1 g L<sup>-1</sup> as positive control according to Zhang and Quantick (1997). Fruits were stored at 4 ± 1 °C for 35 and 16 d for apple and strawberry, respectively. Mold growth of inoculated fruits was measured in term of the decay area and followed by a fungal count every 7 and 4 d in apple and strawberry, respectively.

**2.3.6.2. Determination of fruits tissue deterioration.** Malondialdehyde (MDA) contents were measured according to Hong, Xie, Zhang, Sun, and Gong (2012). A 2 g from fruits tissue was homogenized in 6 mL of 100 mL L<sup>-1</sup> trichloroacetic acid then centrifuged for 15 min at 6000g. The supernatant was collected and 2 mL was mixed with 6 mL of 6 mL L<sup>-1</sup> thiobarbituric acid. The mixture was heated to 100 °C for 20 min, quickly cooled down and centrifuged at 6000g 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:

$$\text{MDA} = [(6.45 \times (A_{532} - A_{600})) - (0.56 \times A_{450})] \quad (2)$$

where: A<sub>532</sub>: Absorbance at 532 nm, A<sub>600</sub>: Absorbance at 600 nm, A<sub>450</sub>: Absorbance at 450 nm and 6.45 as well 0.56 are a consonant.

**2.3.6.3. Determination of weight losses, total soluble solids and titratable acidity.** 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 at the beginning of the experiment an every 7 and 4 d for apple and strawberry, respectively according to A.O.A.C. (2000). The total soluble solids (TSS) and the titratable acidity was determined according to A.O.A.C. (2000).

### 2.3.7. Statistical analysis

The statistical analysis was carried out using SPSS program (ver. 19) with multi-function 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, Torrie, and Dickey (1997).

## 3. Results and discussions

### 3.1. Effect of olive residues extracts incorporated with chitosan on its antimicrobial activity

#### 3.1.1. Antifungal activity

The CH and incorporated CH-OOR coating solutions in different concentrations exhibited strongly antifungal activity by reducing the mycelia yield as shown in Table 1. Surely, the addition of OOR extracts to CH led significantly to increase inhibition activity against tested fungal strains compared with CH solutions only. Generally, CH-OLE recorded higher effect against selected fungal strains at 10 and 20 g L<sup>-1</sup> than CH-OPE giving 39.83 and 58.01%, respectively. Regardless of the coating formulas, the inhibition percentage was evidently higher against *P. expansum* than *R. stolonifer*. Recently, number of studies concluded that the antifungal activity for CH incorporated films with food processing residues extracts such as grape seeds (Rubilar et al., 2013), active shrimp (Arancibia et al., 2014) and blueberry leaf (Yang et al., 2014). However, until now there are no available results about the antifungal activity of CH films incorporated with both OOR extracts so far.

#### 3.1.2. Antibacterial activity

Obtained data perceived that all coating formulas exhibited antibacterial activity against selected food pathogens. Indeed, significant difference ( $p < 0.05$ ) was found between both CH-OLE and CH-OPE as shown in Table 2 and Fig. 1. CH-OLE and CH-OPE at 30 g L<sup>-1</sup> gave the highest inhibition compared with the lower concentrations to be 16.10 and 15.00 mm, respectively, for instance. Obviously, CH-OLE recorded higher inhibition against pathogens than CH-OPE. Also the highest effect of CH-OLE and CH-OPE was observed against *E. coli* O26 to be 20.50 and 18.50 mm, respectively, while no effect occurred against *S. typhi* (Table 2). The antibacterial activity of chitosan film in doubt and questionable in term some author observed chitosan film have antibacterial activity as mentioned by Dutta et al. (2009). However some studies proposed that the chitosan film do not exude any effect or even minor effect against some tested bacterial strains as remarked by Arancibia et al. (2014), and Sun, Wang, Kadouh, and Zhou (2014). Therefore, recent investigation try to improve CH based

**Table 1**

Antifungal activity for chitosan and chitosan incorporated coating solution against tow spoilage fungi strains (Mean ± SD), n = 3.

Coating solution	Conc.	Strain/Inhibition percentage (%) <sup>1</sup>		Mean ± SD
		<i>P. expansum</i>	<i>R. stolonifer</i>	
CH	20 g L <sup>-1</sup>	38.78 ± 0.51bA	24.79 ± 0.54aA	31.78 ± 8.09A
	10 g L <sup>-1</sup>	45.37 ± 1.86bC	34.31 ± 1.14aC	39.83 ± 6.50C
CH-OPE	20 g L <sup>-1</sup>	62.03 ± 0.54bE	54.00 ± 2.65aE	58.01 ± 4.89E
	10 g L <sup>-1</sup>	41.11 ± 0.54bB	31.34 ± 0.54aB	36.22 ± 5.66B
	20 g L <sup>-1</sup>	57.36 ± 2.47bD	50.60 ± 1.44aD	53.97 ± 4.23D

*P. expansum*: *Penicillium expansum*, *R. stolonifer*: *Rhizopus stolonifer*.

a, b, c, . . . : Means with the same letter in the same row are not significant different ( $p > 0.05$ ).

A, B, C, . . . : Means with the same letter in the same column are not significant different ( $p > 0.05$ ).

<sup>1</sup> Inhibition rate was calculated as percentage (%) see Section 2.

**Table 2**  
Effect of antibacterial chitosan coating solution incorporated with different concentrations of olive residues extracts on some tested pathogenic bacterial strains (Mean ± SD), n = 3.

CH-OOR coating solution <sup>1</sup>	Concentration (%)	Strain/Inhibition zone (mm)										Mean ± SD		
		<i>B. cereus</i>	<i>E. coli</i>	<i>E. coli</i> O16	<i>E. coli</i> O26	<i>E. coli</i> O103	<i>E. coli</i> O121	<i>E. coli</i> O157	<i>L. monocytogenes</i>	<i>S. typhi</i>	<i>S. Typhimurium</i>		<i>Staph. aureus</i>	<i>Yersinia</i> spp.
CH-OLE	5 g L <sup>-1</sup>	7.50 ± 0.71	11.50 ± 2.12	7.50 ± 0.71	12.00 ± 0.00	8.50 ± 0.71	11.50 ± 0.71	9.50 ± 0.71	11.00 ± 1.41	-	9.00 ± 1.41	9.50 ± 0.71	12.00 ± 0.00	9.13 ± 3.31A
	10 g L <sup>-1</sup>	9.50 ± 0.71	13.50 ± 2.12	11.50 ± 0.71	13.50 ± 0.71	12.50 ± 0.71	12.50 ± 0.71	11.50 ± 0.71	11.50 ± 2.12	8.00 ± 1.41	11.50 ± 0.71	11.50 ± 0.71	13.50 ± 0.71	11.71 ± 1.80B
	20 g L <sup>-1</sup>	11.00 ± 0.00	14.50 ± 0.71	13.50 ± 0.71	15.50 ± 0.71	17.50 ± 0.71	14.50 ± 0.71	12.50 ± 0.71	12.50 ± 2.12	10.00 ± 0.00	14.50 ± 0.71	13.50 ± 2.12	14.50 ± 0.71	13.67 ± 2.09C
CH-OPE	5 g L <sup>-1</sup>	14.50 ± 0.71	18.50 ± 2.12	17.50 ± 0.71	20.50 ± 0.71	19.50 ± 0.71	15.50 ± 0.71	14.50 ± 0.71	14.50 ± 0.71	12.50 ± 0.71	15.00 ± 1.41	15.00 ± 1.41	15.50 ± 0.71	16.08 ± 2.44D
	10 g L <sup>-1</sup>	10.50 ± 0.71	10.00 ± 0.00	-	11.00 ± 1.41	8.50 ± 0.71	7.50 ± 0.71	10.50 ± 0.71	10.00 ± 0.00	-	10.50 ± 0.71	7.50 ± 0.71	8.50 ± 0.71	7.88 ± 3.81A
	20 g L <sup>-1</sup>	13.50 ± 0.71	11.50 ± 0.71	10.50 ± 0.71	13.50 ± 0.71	12.50 ± 0.71	9.50 ± 0.71	12.00 ± 0.00	11.50 ± 0.71	11.50 ± 0.71	12.00 ± 0.00	9.50 ± 0.71	10.00 ± 1.41	11.46 ± 1.44B
CH-OOR	5 g L <sup>-1</sup>	15.00 ± 1.41	13.50 ± 0.71	11.50 ± 0.71	15.50 ± 0.71	14.50 ± 0.71	12.50 ± 0.71	13.50 ± 0.71	13.50 ± 0.71	13.50 ± 0.71	13.50 ± 0.71	11.00 ± 0.00	11.50 ± 2.12	13.25 ± 1.53C
	10 g L <sup>-1</sup>	16.50 ± 0.71	14.50 ± 0.71	14.50 ± 0.71	18.50 ± 0.71	16.50 ± 0.71	14.50 ± 0.71	14.00 ± 1.41	15.50 ± 0.71	15.00 ± 0.00	14.00 ± 1.41	12.50 ± 0.71	14.00 ± 1.41	15.00 ± 1.66D
	20 g L <sup>-1</sup>	16.50 ± 0.71	14.50 ± 0.71	14.50 ± 0.71	18.50 ± 0.71	16.50 ± 0.71	14.50 ± 0.71	14.00 ± 1.41	15.50 ± 0.71	15.00 ± 0.00	14.00 ± 1.41	12.50 ± 0.71	14.00 ± 1.41	15.00 ± 1.66D

*B. cereus*: *Bacillus cereus*, *E. coli*: *Escherichia coli* O16, *E. coli* O26: *Escherichia coli* O26:H11, *E. coli* O103: *Escherichia coli* O103:H2, *E. coli* O121: *Escherichia coli* O121, *E. coli* O157: *Escherichia coli* O157:H7, *L. monocytogenes*: *Listeria monocytogenes*, *S. typhi*: *Salmonella typhi*, *S. Typhimurium*: *Salmonella Typhimurium*, *Staph. aureus*: *Staphylococcus aureus*, *Yersinia* spp.: *Yersinia* spp.

A, B, C, . . . : Means with the same letter in the same column are not significant different (p > 0.05).

- Not detected.

<sup>1</sup> Chitosan film incorporated with olive residues extracts.

film functional properties (Siripatrawan & Harte, 2010; Sun, Wang, Kadouh, & Zhou, 2014).

### 3.2. Physical properties of chitosan edible films incorporated with olive oil residues extracts

#### 3.2.1. Film appearance

The incorporated CH film surface was photographed and shown in Fig. 2. The CH film at 20 g L<sup>-1</sup> exhibited a pale yellow color, smooth, glossy, without visible pores and cracks. However, no changes were observed in texture and odor property when add both OOR. Otherwise, the color of incorporated films was more opercula especially CH-OPE. The CH or CH-based films were transparent, thin, smooth appearance and easy to handle (Pastor, Sánchez-González, Chiralt, Cháfer, & González-Martínez, 2013; Velickova, Winkelhausen, Kuzmanova, Alves, & Moldão-Martins, 2013).

#### 3.2.2. Film thickness

The thickness of CH films incorporated with OPE and OLE at various concentrations were tabulated in Table 3. Significant difference (p < 0.05) was observed in thickness between both CH-OLE and CH-OPE at 20 g L<sup>-1</sup> and pure CH film due to OOR phytochemicals. Otherwise, no significant difference (P > 0.05) was observed between CH films and CH incorporated films with OLE and OPE at 10 g L<sup>-1</sup> (Table 3). These results are in agreement with Rubilar et al. (2013), Velickova et al. (2013) who proposed that incorporation of grape seeds extracts or beeswax to CH solution increased the film thickness significantly.

#### 3.2.3. Films solubility

OOR extracts presence reduced water solubility of CH based films as can be seen in Table 3. The CH film showed the highest film solubility of 26.40%. The increasing of OOR especially OLE decreased the solubility significantly. The CH-OLE 20 g L<sup>-1</sup> exhibited the lowest solubility. No significant difference (P > 0.05) of solubility between CH and CH-OPE 10 g L<sup>-1</sup> (and CH-OLE 10 g L<sup>-1</sup> and CH-OPE 20 g L<sup>-1</sup>) was observed. Similar results were obtained when hydrophobic agents were incorporated into CH film such as tocopherol, cinnamon essential oil and grape seeds extracts (Martins, Cerqueira, & Vicente, 2012). It is also important to remark that an incremental rate in film solubility could affect the integrity of those films in some technological application.

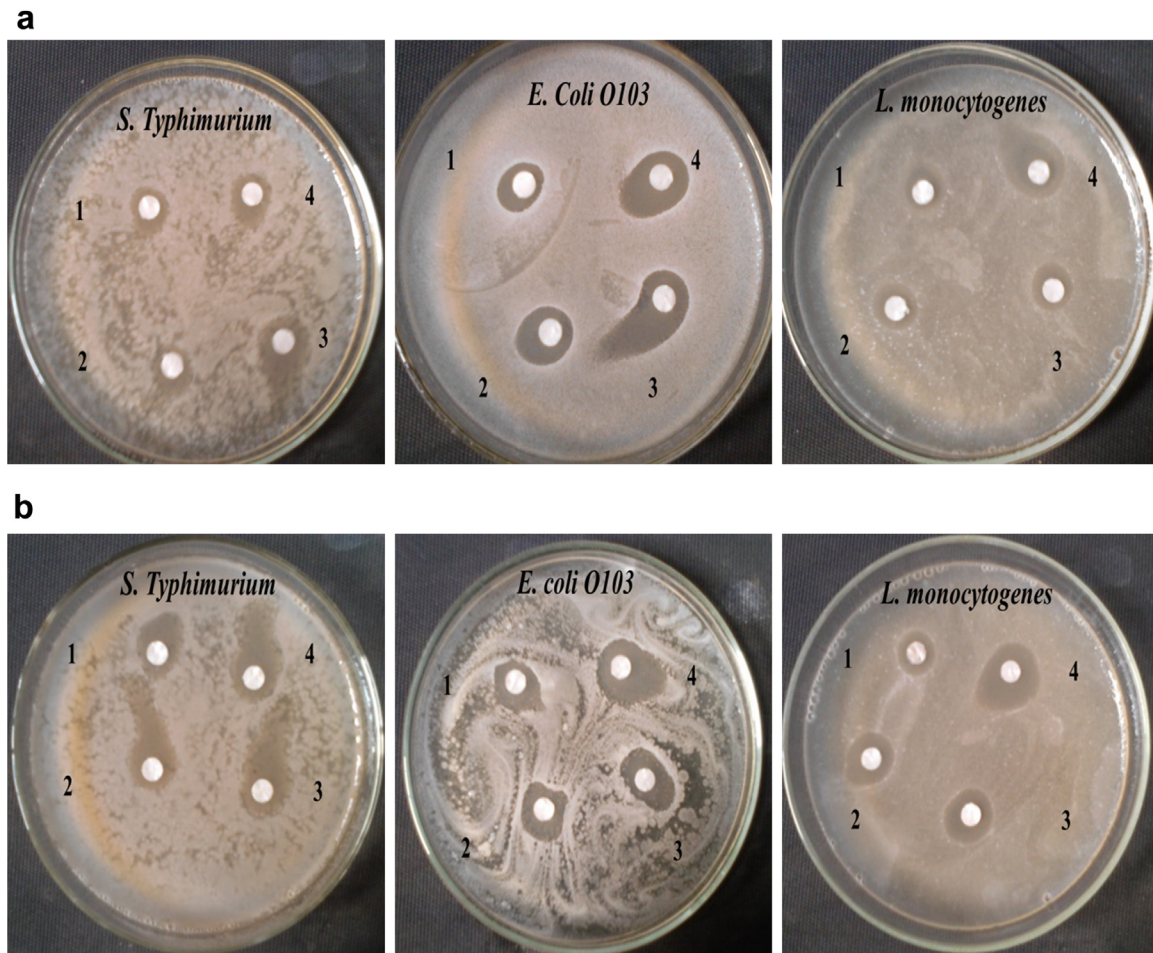
#### 3.2.4. Microbiological quality attributes

##### 3.2.4.1. Effect of CH-incorporated coating formulas on the growth of *P. expansum* and *R. stolonifer* during cold storage of apple and strawberry fruits.

Generally, the infected area was gradually increased with increasing the storage periods as shown in Fig. 3. Regardless the coating treatments, the initial area were 3 and 2 mm in apple and strawberry fruits, respectively. Those areas exhibited circular infection to be 13.28 and 15.00 mm at the end of storage in apple and strawberry, respectively. Indeed, the decayed areas of both coated apple and strawberry fruits were significantly reduced when compared with the uncoated fruits. Subsequently, the early signs of mold development in uncoated apple and strawberry appeared after 7 and 4 d of storage, respectively.

Consequently, the coated fruits with CH-OLE 20 g L<sup>-1</sup> recorded the highest decrease fruit in decayed area than either CH or WW-TBZ coated and uncoated fruits. Obviously, the highest observed areas were 25.33 and 26.50 mm in uncoated apple and strawberry at the end of storage, respectively. On the other hand, the lowest observed areas were 7.33 and 8.00 mm in coated apple and strawberry with CH-OLE 20 g L<sup>-1</sup>, respectively. The CH and CH-OOR





**Fig 1.** a. Antibacterial activity for CH-OLE 1, 2, 3 and 4 for 5, 10, 20 and 30 g L<sup>-1</sup> coating formulas against some selected pathogenic bacterial strains, as exemplary. Data were tabulated in Table 2. b. Antibacterial activity for CH-OPE 1, 2, 3 and 4 for 5, 10, 20 and 30 g L<sup>-1</sup> coating formulas against some selected pathogenic bacterial strains, as exemplary. Data were tabulated in Table 2.

were more effective than the commercial coating material of WW-TBZ on growth of fungal strains as revealed shown in Fig. 4. These motivated results could encourage the food handlers to replace the chemical coating materials with the presented coatings formulas of the current study. However, these coatings materials were more effective on apple than strawberry. This might be due to the different surface characteristics allowing apple to hold more coating materials on the surface than strawberry. Otherwise, solidifying agent should be included in strawberry coating materials formulas (further studies). These results are

**Table 3**  
Physical properties of Chitosan edible films incorporated with Olive oil residues extracts (Mean ± SD), n = 3.

Film formulas	Properties	
	Thickness (mm)	Water solubility (%)
CH <sup>1</sup>	0.092 ± 0.002A	26.40 ± 0.67A
CH-OLE 10 g L <sup>-1</sup>	0.097 ± 0.001A	21.63 ± 0.98C
CH-OPE 10 g L <sup>-1</sup>	0.093 ± 0.002A	24.13 ± 1.93B
CH-OLE 20 g L <sup>-1</sup>	0.129 ± 0.009C	17.82 ± 1.22D
CH-OPE 20 g L <sup>-1</sup>	0.106 ± 0.008B	20.63 ± 0.80C

A, B, C, . . . : Means with the same letter in the same column are not significant different ( $p > 0.05$ ).

<sup>1</sup> CH film was prepared as 20 g L<sup>-1</sup> concentrate.

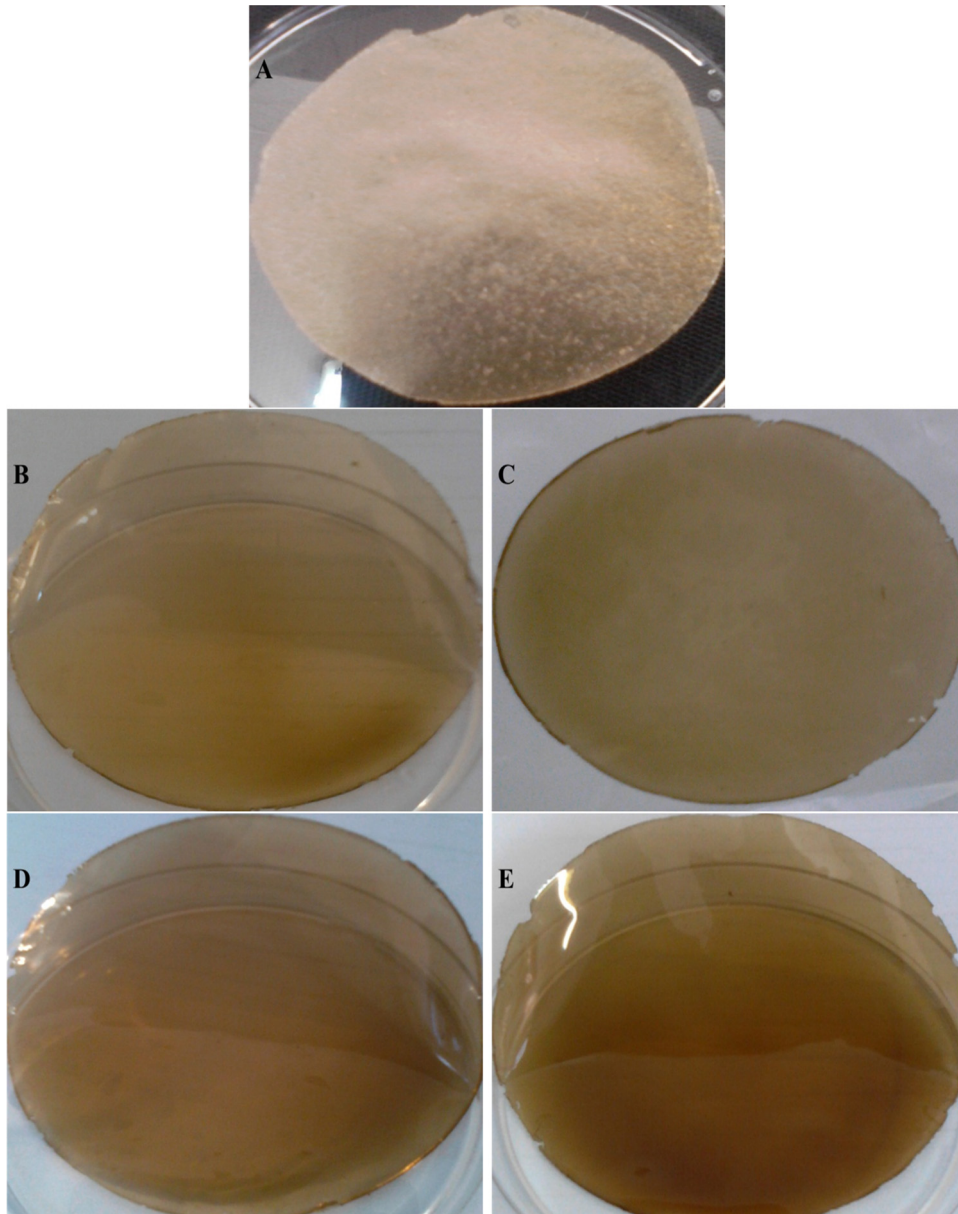
complimentary to those of Park, Stan, Daeschel, and Zhao (2005) who reported significant antimycotic activity of methylcellulose and CH composite films incorporated sodium benzoate or potassium sorbate.

The fungal count of *P. expansum* and *R. stolonifer* as logarithmic number (log CFU g<sup>-1</sup>) is presented in Fig. 5. The fungal count of *P. expansum* and *R. stolonifer* was rapidly increased during the cold storage in uncoated fruits. The lowest fungal count was recorded in coated fruits with CH-OLE 20 g L<sup>-1</sup> or CH-OPE 20 g L<sup>-1</sup>. It is bearing in mind that the growth of fungal strains on the surface or the cross section of the fruit being one of the causes of corruption for these fruits and malformed appearance. These findings are confirmed recently (Park et al., 2005; Rodríguez, Ramos, & Agulló, 2003), who reported that CH coating delayed the growth of *Rhizopus* sp. and *Cladosporium* sp. in strawberry and pizza. Moreover, the inhibition effectiveness of CH 10 g L<sup>-1</sup> formulas was noticed against *P. expansum* and *B. cinerea* growth on apples (Shao, Tu, Tu, & Tu, 2012).

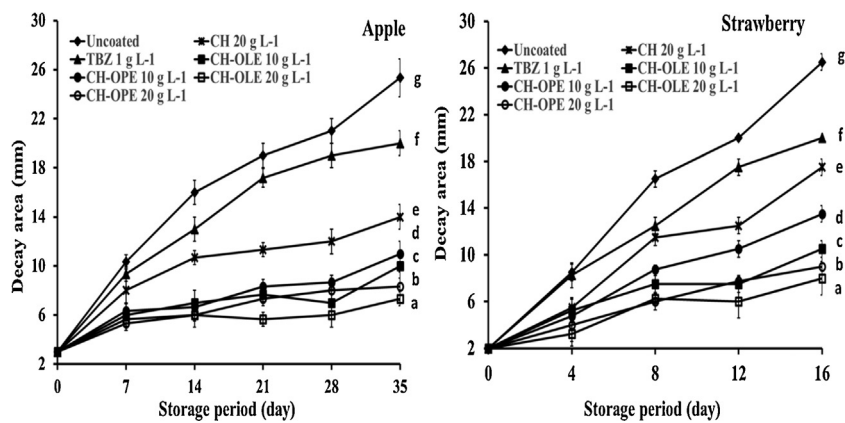
### 3.3. Freshness quality attributes

#### 3.3.1. MDA contents

The MDA content was used as a direct indicator of cell membrane injury and index of cell oxidative damage (Xu et al., 2009). As shown in Fig. 6. MDA contents in apple and strawberry pulp increased



**Fig. 2.** Over-section photos for surface of CH and CH incorporated films; (A): CH 20 g L<sup>-1</sup>, (B): CH-OLE 10 g L<sup>-1</sup>, (C): CH-OPE 10 g L<sup>-1</sup>, (D): CH-OLE 20 g L<sup>-1</sup> and (E): CH-OPE 20 g L<sup>-1</sup>, (Mean ± SD), n = 3.



**Fig. 3.** Effect of CH incorporated coating solution on fungal growth of artificially infected *P. expansum* in apple and *R. stolonifer* in strawberry fruits during cold storage at 4 ± 1 °C, (Mean ± SD), n = 3.



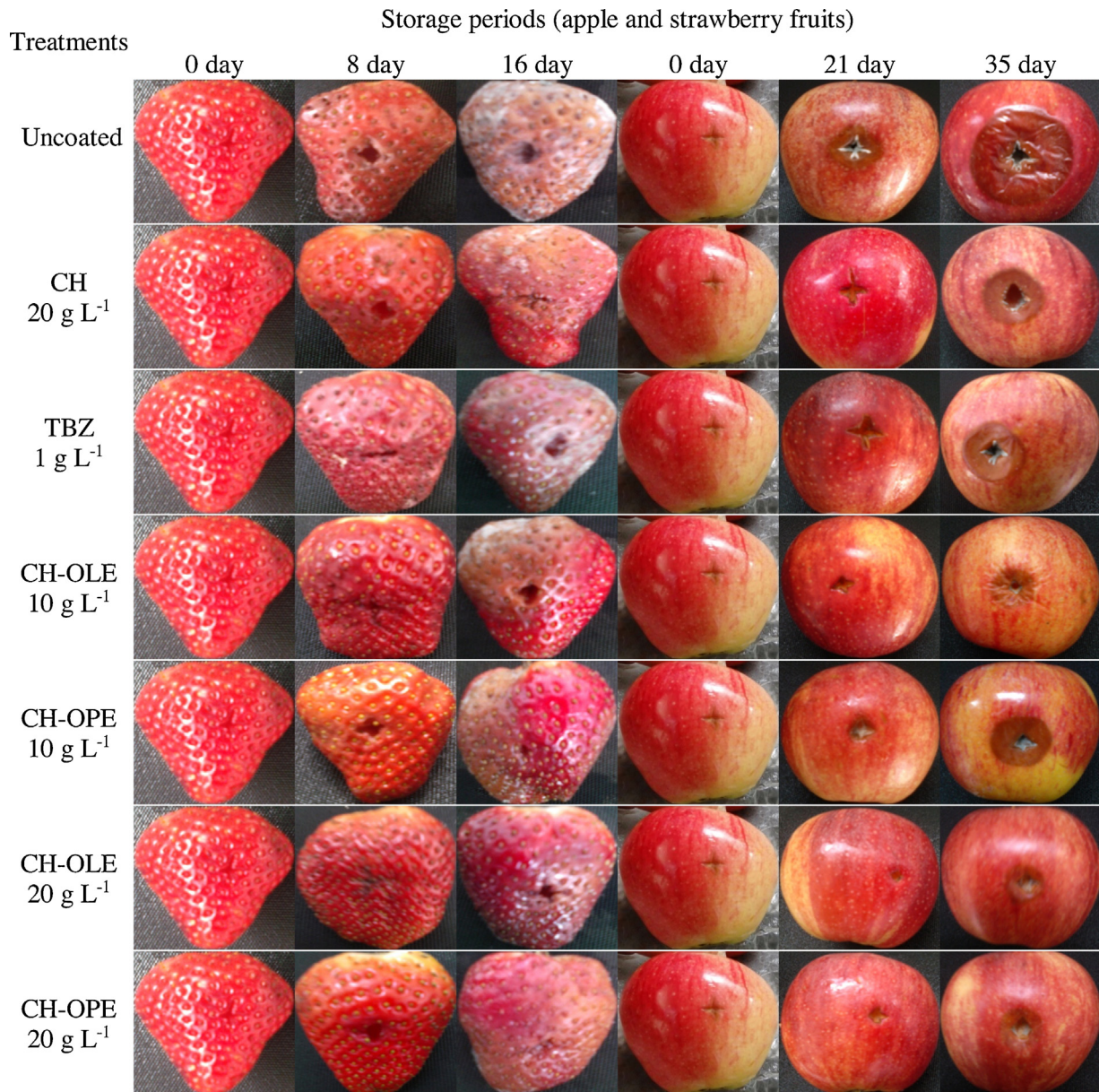


Fig. 4. Effect of CH and CH-OOR with different concentrations on *P. expansum* and *R. stolonifer* growth in apple and strawberry fruits, respectively during storage at 4 ± 1 °C. (Mean ± SD), n = 3.

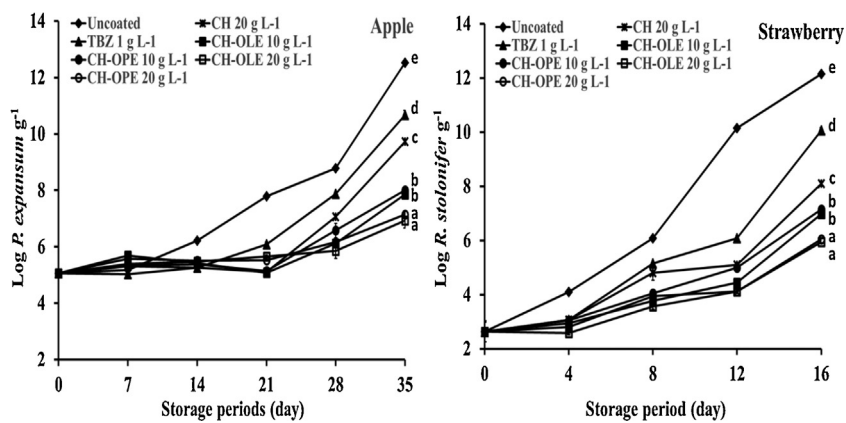


Fig. 5. Effect of coating with CH, WW-TBZ and CH-incorporated coating solution on apples infected by *P. expansum* and strawberry infected by *R. stolonifer* during cold storage at 4 ± 1 °C, (Mean ± SD), n = 3.

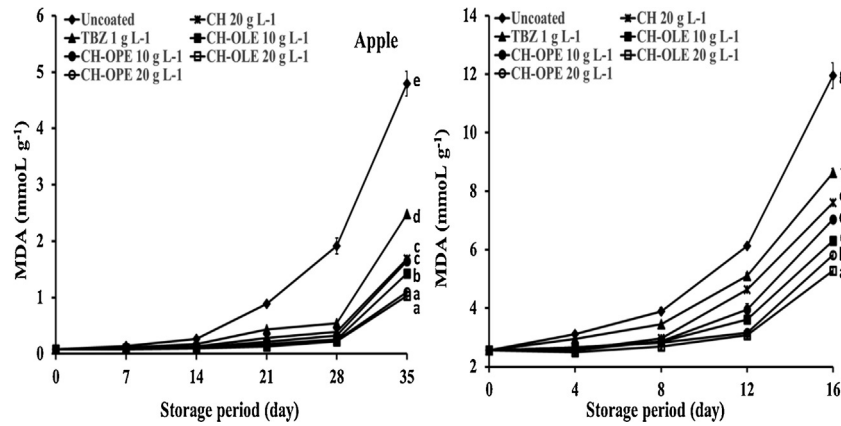


Fig. 6. Monitoring of MDA for coated and uncoated apple and strawberry fruits with CH incorporated coating solution during cold storage at  $4 \pm 1 \text{ }^\circ\text{C}$ , (Mean  $\pm$  SD),  $n = 3$ .

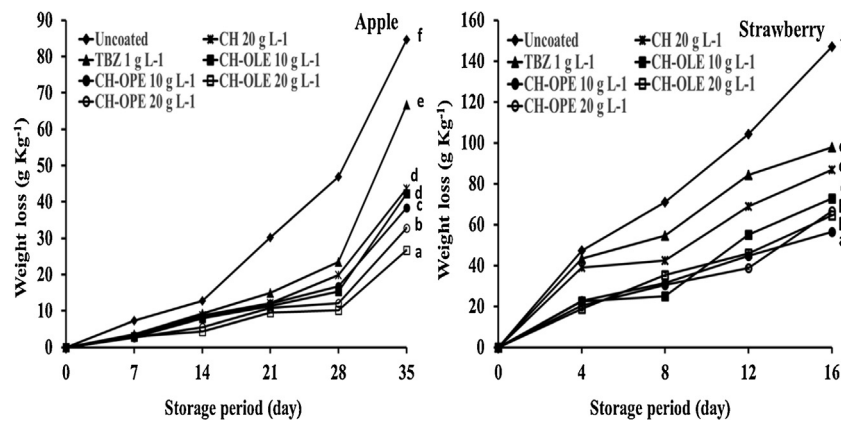


Fig. 7. Changing of weight loss for coated and uncoated apple and strawberry fruits with different formulas during cold storage at  $4 \pm 1 \text{ }^\circ\text{C}$ , (Mean  $\pm$  SD),  $n = 3$ .

during cold storage period. The initial and the final MDA in apple and strawberry was (0.08 and 2.55) and (2.02 and 7.51)  $\text{mmol g}^{-1}$ , respectively, for instance. Obviously, the uncoated fruits recorded significantly higher MDA than the coated fruits. To enumerate that, the MDA in uncoated apple and strawberry were 1.34 and 5.52  $\text{mmol g}^{-1}$ , respectively. However these values reached in apple and strawberry coated with CH-OLE  $20 \text{ g L}^{-1}$  to 0.27 and 3.22

$\text{mmol g}^{-1}$ , respectively. The dramatically increase of MDA in uncoated fruits especially strawberry, resulted soft and pale tissues when compared with apple fruits. However, coating treatment improved the membrane integrity and increased the keeping quality of kept fruits. Some recent studies concluded that coating with CH reduced the increasing of MDA in grapes (Yinzhe & Shaoying, 2013), sweet pepper (Xing et al., 2011) and apple (Shao et al., 2012).

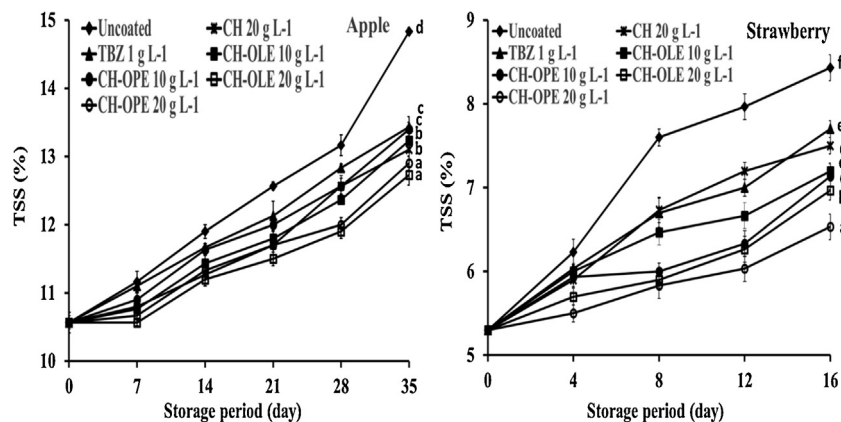


Fig. 8. Varying of TSS for coated and uncoated apple and strawberry fruits with CH incorporated coating solution during cold storage at  $4 \pm 1 \text{ }^\circ\text{C}$ , (Mean  $\pm$  SD),  $n = 3$ .



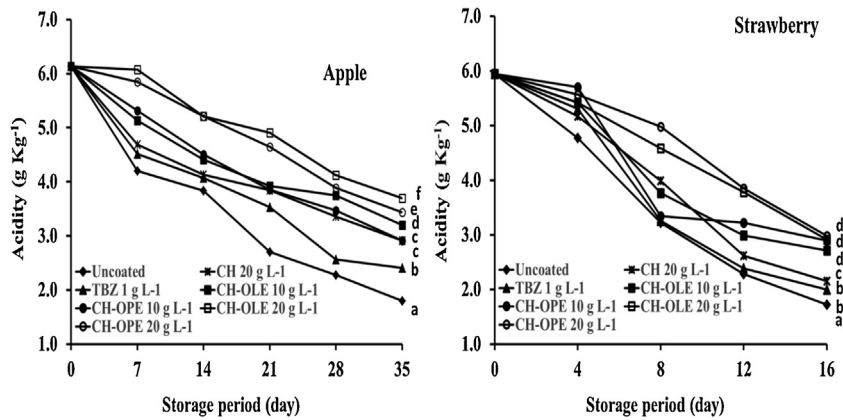


Fig. 9. Fluctuating of titratable acidity for coated and uncoated apple and strawberry fruits with CH incorporated coating solution during cold storage at  $4 \pm 1$  °C, (Mean  $\pm$  SD),  $n = 3$ .

### 3.3.2. Weight loss

Surely, coating formulas reduced significantly ( $p < 0.05$ ) weight loss in both fruit compared with uncoated fruits as portrayed in Fig. 7. The highest loss was recorded in uncoated apples after 35 d reached to  $85 \text{ g kg}^{-1}$ , while this rate reached to  $147 \text{ g kg}^{-1}$  in uncoated strawberries after 16 d. The lowest weight loss in strawberry and apple fruits at the end of storage period was  $56.3$  and  $26.6 \text{ g kg}^{-1}$  using CH-OPE  $10 \text{ g L}^{-1}$  and CH-OLE  $20 \text{ g L}^{-1}$ , respectively. The formed CH film on surface of coated fruits delayed the migration of moisture. These coating strategies to reduce the weight loss during storage and its concept were used in strawberries (Gol et al., 2013). Nevertheless, Shao et al., 2012 used CH for apple coating with pullulan).

### 3.3.3. Total soluble solids

Clearly, TSS levels at the beginning of storage were  $10.56$  and  $5.30\%$  in apple and strawberry, respectively. They are increased at the end of storage in both uncoated and coated fruits to be  $13.4$  and  $7.5\%$  in apple and strawberry, respectively as presented in Fig. 8. The increases in TSS during storage were mentioned by Rivera-López et al. (2005), due to the breakdown of starch into soluble sugars or the hydrolysis of cell wall polysaccharides. Controversial, uncoated fruits exhibited TSS higher than coated fruits with CH or CH-OOR which reached to  $12.36$  and  $8.43\%$  in apple and strawberry fruits, respectively. Coated apple with CH-OLE  $2\%$  exhibited the lowest incremental rate in TSS contents to be  $12.73\%$ . Also, 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 and fruits coating. It may seem to work as pores barrier reducing  $\text{O}_2$  exchange.

### 3.3.4. Titratable acidity

Slow decremental rate of TA in coated fruits rather than coated ones was observed Fig. 9. Combined CH with OLE or OPE was significantly reduced the acidity reduction compared to uncoated fruits and CH as well as WW-TBZ formulas. Generally, no significant difference ( $p > 0.05$ ) was showed between coated strawberry with CH-OLE  $20 \text{ g L}^{-1}$  or CH-OPE  $20 \text{ g L}^{-1}$ , hence both of them recorded the lowest decremental acidity rate to be  $2.9$  and  $3.0 \text{ g kg}^{-1}$ , respectively. For explanation, the changes in acidity are significantly affected by the rate of metabolism especially respiration, which consumed organic acids and therefore decline the acidity during storage, this also may cause fruit senescence. Retention of acidity decreases has been reported previously in coated strawberry fruit

with CH-beeswax coatings (Gol et al., 2013). Moreover, in coated apple fruits with CH films (Shao et al., 2012).

## 4. Conclusion

The results of the present study asserted that the incorporation of OOR into CH was improved its filmogenic properties. Also, the coatings of CH or CH-OOR have a beneficial impact on the quality retention of cold storage apple and strawberry fruits especially when coated by CH-OLE  $20 \text{ g L}^{-1}$ . The use of OOR also maintained lower activities of cell wall deterioration as calculated by MDA. Likewise, the incorporation of OOR to CH enhanced its antifungal activity against *P. expansum* and *R. stolonifer* counted and decay area. Hence, coatings of apple and strawberry fruits with CH-OOR may be useful for improving postharvest quality and shelf-life comparing to CH only or WW-TBZ, applying it commercially is indigence.

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