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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^{-1} 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.

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-Uribe, 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^{-1} to be 9.788 kg Ha^{-1} (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; Terpinc, Čeh, Ulrih, & Abramovič, 2012), antimicrobials (Keskın, 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® 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

a Olive (***Olea europaea*var.*Kronakii***) residue leaves and olive pomace were obtained from Cairo for Oil Industry Co., industrial zone, 6th October city, Egypt.
b Fresh apple fruits (***Malus. domestica*var.*Anna***) were obtained from an Alexandria Agriculture Farm, 70 km Cairo-Alexandria desert road, Egypt.
c 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 ± 1 °C until use. On the other hand, both olive leaves and pomace were individually mixed with ethanol 800 mL L^{-1} 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^{-1} 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^{-1} CH was dispersed in an aqueous solution of glacial acetic acid (5 mL L^{-1}, 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^{-1} NaOH. Subsequently, glycerol 16 mL L^{-1} was added as a plasticizer (Sánchez-González et al., 2011). The OLE and OPE 10 and 20 g L^{-1} (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 8000*g* 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^{-1}) on antifungal properties of CH 20 g L^{-1} 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 10^5 spore mL^{-1} of *P. expansum* and *R. stolonifer*. The flasks were incubated at 28 ± 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:

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

where: DW_{utf}: dry weight of untreated fungal strain and DW_{etf}: 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^{-1}) 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^{-1} then poured in sterilized petri dishes. Consequently, CH-OOR was sterilized by 0.45 μm filters (Minisart®, 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).