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

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quercetin (PubChem CID: 5280343)
2
6-dichlorophenol-indophenol (PubChem CID: 23697355)
chitosan (PubChem CID: 3086191) and
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 $\leq$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.; Paini 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$^{\bullet}$), 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, 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, Sinanoglou, 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 (10^5 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 (1and 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 warp 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} Na_2CO_3 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^{-1}dw) using the following equation based on the calibration curve:

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

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} $AlCl_3$ 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^{-1}dw) which was calculated using the following equation based on the calibration curve:

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