Influence of postharvest application of chitosan combined with ethanolic extract of liquorice on shelflife of apple fruit

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RESEARCH ARTICLE

Influence of postharvest application of chitosan combined with ethanolic extract of liquorice on shelflife of apple fruit

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Abstract

Background Edible coatings are useful method that applied to preserve postharvest quality of production. The coatings can extend the shelf life of products and inhibit microbial growth. Chitosan based coatings are one of the best methods to prolong fruit and vegetable shelf life. The antimicrobial and other properties of chitosan are developed when it is combined with other functional ingredients.

Methods The effectiveness of chitosan, ethanolic extract of liquorice (LE) and complex of chitosan-liquorice extract (CHLE) was evaluated for controlling blue mold and extending shelf life in apples. The fruits were coated with chitosan(1.0%), LE (62.5 mg/ml) and CHLE coating, and stored at 25 °C. Quality properties of fruit (such as weight loss, firmness, total soluble solid content(TSS), titrable acidity and pH) and decay incidence were assessed on 0,1,4,7 and 14 days of incubation, respectively.

Results The results of experiments indicated that minimum of water loss(3.8%), TSS(14.53) and firmness(5.6 kg/cm^2) were in CHLE coated apples. In addition, this coating significantly inhibited *penicillium expansum* during the storage and the lowest decay incidence was for apples coated with CHLE(29 mm). Chitosan and LE coating retarded undesirable changes during postharvest storage and inhibited decay incidence compared with uncoated samples. There was no significant difference ($p \leq$ 0/05) between treatments and control overtime in terms of titrable acidity and pH levels.

Conclusions The results reported here indicate importance and efficacy of CHLE coating in extending shelflife and reduction of postharvest losses of apple in storage time.

Keywords Postharvest losses · Edible coating · Liquorice extract · Chitosan

Introduction

According to a report by the food and agriculture organization global(FAO) the average of postharvest losses in Europe, North America and Oceania is 29% and in industrialized Asia, South East Asia, Africa and Latin America is 38% [1–3]. Various methods are used to reduce postharvest losses during the supply chain of fresh produce. However, the increasing concern about the potential harms of synthetic chemical compound on human health and environment and also the development of resistant pathogens are the most important reasons to find alternative strategies [4].

Penicillium expansum is responsible for blue mold, one of the most important agents of postharvest losses in apple fruit. In addition, *penicillium expansum* causes patulin generation, a mycotoxin that causes genotoxicity in mammalian cells [5]. In recent years, the utilization of edible coatings as a safe and beneficial strategy has received a lot of consideration to

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decline postharvest losses. [6]. Chitosan is a safe natural polymer composed of 2-amino-2deoxy-β-D-glucose [7] which is generated from deacetylation of chitin, [8], fungi and insects [9]. It has antibacterial, antifungal and antiviral activities and due to having applicable properties, it has earned a great deal of consideration in various industries [8]. It seems that the antimicrobial and other properties of chitosan are developed when it is combined with other functional ingredients [10]. The water soluble chitosan controlled the mycelium growth and spore germination of *P.expansum* in a concentration-dependent manner, both in vivo and in vitro conditions. [11]. Furthermore, numerous studies indicated that antimicrobial and other properties of chitosan increases when chitosan is combined with other antimicrobial agents in fruits like strawberry [12], table grape [9], tomato [13] and broccoli [14].

The liquorice plant is a herbaceous perennial legume native to Mediterranean, southern of Russia and Asia. [15]. The liquorice extract has antimicrobial activities against *Candida albicans, Bacillus subtilis, Escherichia coli* [16], *Aspergillus flavus*, *Alternaria alternate*, *Erwinia herbicola* and other pathogens [17].

The targets of our study were to examin the influence of chitosan_liquorice extract edible coating on shelflife and reduction of postharvest losses of apple fruit as a safe replacement strategy in storage time.

Material and methods

Preparation of plant extract

The liqourice root was purchased from medicinal herbs market in Tehran. The roots were finely powdered by using grinder (Bosch MKA 6003, Slovenia). A total of 50 g liqourice powder along with 500 ml of ethanol (80%) were poured into a flask and placed in a shaker incubator (model innova ™ 4340, USA) at 40 °C for 24 h with a rate of 150 round/min. Then, the extracted liqourice was filtered using whatman filter paper N.2 and concentrated using a rotary evaporator (IKA RV10, Japan) at 40 °C for 1 h. Finally, it was completely dried in oven (Gallencamp, UK) at 50 °C for 24 h and stored in 4°C.

Preparation of chitosan

Chitosan solution (1.0%) was prepared by dissolving 5 g chitosan powder (Sigma Aldrich,USA) in 250 ml of distilled water containing 10 ml glacial acetic acid (Merck, Germany) and 0.05% tween 80 (Merck, Germany). The solution was shaken constantly for 24 h using a shaker incubator at 25 °C and 150 rpm. Then, the pH was adjusted to 5.2 with 4 N NaOH and more distilled water was added until the volume became 500 ml. Ultimately, the solution was sterilized for 15 min with an autoclave at 121 °C and 15 psi pressure.

Fungal culture

P.expansum was attained from university of Tehran, Abureyhan campus. The fungi were cultured on potato dextrose agar (PDA) at 25 °C for 7 days.

Preparation of the fungal suspension

For preparation of a homogenous suspension, Macfarland standard was used. The prepared suspension contained 1.5×10^8 (CFU/ml).

Fruit

Apples, CV Damavand were purchased from a local market in Tehran. The fruits were chosen based on the equality of size, shape, color and loss of wounds and fungal disease. For antifungal investigation, using sterilized peg, two wounds (3 mm wide and 5 mm deep) were made on equatorial two side situation of each fruit and for the quality survey of fruit, the whole fruit was used.

Treatment

The 24 fruits were separated into 4 groups randomly, in 2 series for microbial analysis and shelf life analysis. Chitosan (1.0%), extract (62.5 g/l), chitosan combined with extract and the apples dipped into distilled water were considered as group (T1), group (T2), group (T3) and control group, respectively. At first, the samples were dipped into solutions for 5 min and left to dry for 1 h at room temperature. Next, 20 μL of conidia suspension of *P.expansum* was inoculated in each wound and after that packed into a plastic box, ultimately incubated at 25 °C and 95–90% moisture for 14 days.

In vitro antifungal activity of liquorice extract on *P.expansum*

Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial agent that prevents visible growth of a microorganism after a 24 h incubation [18]. MIC was determined by microdilution method on 24 sink microplates. One millimeter of sterilized potato dextrose broth (PDB) was poured into sinks, and then 1 mL of ethanolic extract of liquorice (1 g/l) was added to the first sink. Next, 1 mL of the the first sink was transferred to the next sink and 1 mL of the last sink contents was thrown away. Then, 100 μL of fungal suspension was added to each sink into a sterile laminar flow cabinet. PDB without extract was used for the control group. The microplates were incubated at 25°c for 48 h. This experiment was implemented twice for the fungi.

The MIC was determined as the lowest concentration of extract where there is no turbidity and visible growth.

Measurement of mycelial radial growth

Autoclaved PDA medium along with chitosan (1 mg/l), LE (62.5 mg/ml) and mixture of this solution were poured into a sterile petri dish (9 cm). Mycelial plugs (4 mm) from 7-day-old cultures of *P. expansum* were placed on the center of the petri dish and incubated at 25 °C. PDA plate was used as control and each treatment was in triplicate in 2 series. The inhibitory effect of mycelial growth was determined by measuring colony diameter on days 0, 1, 3, 5 and 7.

Evaluation of shelf life properties

Rate of weight loss

Weight loss was evaluated according to Vieira et al. (2016) by weighing all fruits with a digital balance (Mettler AE200, England) at the beginning of the experiment (0 day) and at regular intervals of sampling [1, 4, 7]. The percentage of weight loss was calculated by the following formula:

$$\text{weight loss}\ (\%) = (\text{W1}-\text{W2})/\text{W1} \times 100$$

Where, w1 is the initial fruit weight and w2 is the fruit weight at sampling days.

Firmness

Firmness is the main feature of fruit quality. For measuring the firmness of apples, at first, the peel was removed by a sharp knife in the equatorial situation of each fruit, then the firmness was measured using a penetrometer (model GY-3, china) with an 8 mm probe, with values reported per kg/cm^2.

pH

The pH values were determined using a pH meter (Crison, Spain). At first, the instrument was calibrated by the pH of 4, 7 and 9 buffer. The electrode was then immersed in the fruit juice.

Statistical analysis

We utilized R software (R-3.1.0 version) for data analysis. All data were considered by kruscal-wallis test and multiple comparison test. The p value representing a significant difference was less than 0.05.

Result and discussion

In vitro antifungal activity of liquorice extract on *P.expansum*

The first effect of microbial growth controling was detected at concenteration of 62.5 mg/ml and increasing the concentration of the LE improved the rate of inhibition. According to MIC definition, the minimum inhibitory concentration of ethanolic extract of liquorice was determined at a concentration of 500 mg/ml. [19] reported that Glabridin, active ingredient of *liqourice extract* had antifungal activity against some molds and a concentration of 31.25–250 μg/mL could completely inhibit resistant mutants of *Candida albicans*. [19]

The effect of chitosan, LE and CHLE on mycelial radial growth of *P.expansum* on PDA

The mycelium completely covered the control plate after 3 days of incubation. In contrast, chitosan and the CHLE showed a significant inhibitory effect against *P.expansum* compared to controls. LE reduced the mycelial growth of *P.expansum*, though there was no significant difference between the extract and control. The result of this trial are shown in Fig. 1.

The effect of chitosan, LE and CHLE on shelf life properties

At the end of the storage time, the lowest (3.8%) loss weight in samples was obtained for CHLE coated apples and the highest loss weight was for uncoated samples (7.89%). A previous study showed that the weight loss was related to respiration processes and loss of water. Therefore, CHLE coating acted like a barrier and reduced the evaporation of moisture [20]. Chitosan-glucose complex coating decreased weight loss in table grape [9], compared with chitosan and glucose alone. Appling of chitosan-glucose complex restrict weight loss of shiitake mushroom [21].

The present study indicated that postharvest treatment with CHLE edible coating can reduce weight loss. Fruit firmness increased during the holding time. At the end of the storage, the lowest and highest values of firmness were 5.6 and 6.9 kg/cm^2 for CHLE treatment and controls, respectively. In our experiment, CHLE coating was more effective to maintain texture compared with uncoated apples. The firmness of blueberries increased within the first 12 days of storage and the combination of chitosan and *Aloe vera* fraction declined the rate of firmness [20]. This growth could be related to weight losses. However, chitosan with LE treatment significantly reduced fruit firmness during the storage time compared with controls, chitosan and extract alone.

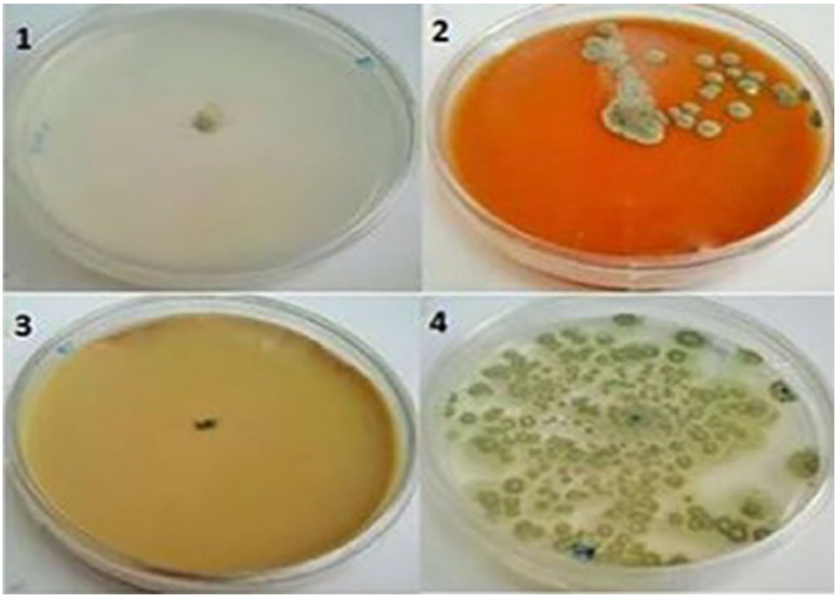

Fig. 1 The effect of chitosan (1), LE (2), CHLE (3) on *P.expansum* after 7 days of incubation in 25°c. unamended plate served control (4)

Total soluble solid content increased over the time of the storage. This is probably due to water loss that led to concentration of the apple sugar within the period of the storage. The uncoated apple presented the highest value (17.5) of TSS at the end of storage. Further, the lowest value (14.53) of TSS in 7 days was recorded for samples coated with chitosan-extract. Hong et al (2012) indicated that the TSS of control and 0.5% chitosan coated guava increased significanty while fruits coated with 1.0 and 2.0% showed a negligible increase during storage [22]. The results of TSS measurement are presented in Table 1.

Titrable acidity decreased over the time across all of the samples, as organic acids were consumed by respiration to support the normal activities of life during the storage. Hong et al. (2012) concluded that the decrease of values titratable acidity was directly in related to the chitosan concentrations and guava treated with 2.0% had higher titratable acidity compared to the other treatments [22]. Ali et al (2011) demonsterated that the highest values ofTA observed in the 2.0% chitosan coated papaya after storage time, while the lowest values were recorded in the uncoated fruit followed by 0.5% chitosan concentration [23]. In our experiment, coatings did not have any effect on TA levels and there was no difference between the treatments and control. It suggested that chitosan, LE and mixture of them in applied concentration did not have any effect on respiration of samples.

Table 1 The effect of chitosan, LE and complex of CHLE coating on the weight loss, firmness, total soluble solid content, titrable acidity and pH of apples stored at 25 °C

Treatment	Loss weight(%)	Firmness(kg/cm^2)	TSS(Brix)	TA	pH
0 day storage					
Chitosan	0.06 ± 0.01	5.30 ± 0.15	13.96 ± 0.20	0.05 ± 0.00	4.3 ± 0.05
LE	0.06 ± 0.02	5.20 ± 0.20	14.21 ± 0.20	0.05 ± 0.00	4.3 ± 0.05
CHLE	0.03 ± 0.00	5.41 ± 0.20	13.23 ± 0.10	0.05 ± 0.00	4.3 ± 0.00
Control	0.13 ± 0.02	5.43 ± 0.15	14.3 ± 0.20	0.04 ± 0.00	4.3 ± 0.05
1 day storage					
Chitosan	0.62 ± 0.06	5.50 ± 0.10	13.36 ± 0.30	0.055 ± 0.00	4.3 ± 0.10
LE	0.64 ± 0.02	5.50 ± 0.10	13.53 ± 0.10	0.056 ± 0.00	4.3 ± 0.00
CHLE	0.43 ± 0.01	5.40 ± 0.05	14.10 ± 0.20	0.05 ± 0.00	4.3 ± 0.05
Control	*0.87 ± 0.00*	5.90 ± 0.10	14.8 ± 0.20	0.05 ± 0.00	4.4 ± 0.10
4 day storage					
Chitosan	4.29 ± 0.22	5.80 ± 0.15	14.26 ± 0.20	0.03 ± 0.02	4.4 ± 0.10
LE	4.14 ± 0.02	5.80 ± 0.05	14.34 ± 0.20	0.05 ± 0.00	4.5 ± 0.10
CHLE	2.73 ± 0.05	5.43 ± 0.15	14.11 ± 0.20	0.05 ± 0.00	4.3 ± 0.05
Control	5.98 ± 0.50	6.90 ± 0.20	16.54 ± 0.15	0.04 ± 0.00	4.5 ± 0.10
7 day storage					
Chitosan	5.57 ± 0.22	5.80 ± 0.20	15.12 ± 0.10	0.046 ± 0.00	4.5 ± 0.05
LE	5.39 ± 0.20	5.80 ± 0.20	15.13 ± 0.10	0.049 ± 0.00	4.4 ± 0.00
CHLE	3.80 ± 0.10	5.60 ± 0.10	14.53 ± 0.10	0.05 ± 0.00	4.4 ± 0.10
Control	7.89 ± 0.50	6.90 ± 0.02	17.54 ± 0.20	0.04 ± 0.00	4.6 ± 0.00

Table 2 The effect of chitosan, LE and CHLE on lesion diameter

Treatment	Lesion diameter(mm)
0 day	
Chitosan	0
LE	0
CHLE	0
Control	0
1 day	
Chitosan	*0*
LE	0
CHLE	0
Control	0
4 day	
Chitosan	13.66 ± 0.57
LE	14.66 ± 0.5
CHLE	11.5 ± 0.5
Control	18.66 ± 1
7 day	
Chitosan	23 ± 2
LE	22.66 ± 0.5
CHLE	20.66 ± 0.5
Control	25.66 ± 0.5
14 day	
Chitosan	38 ± 2
LE	43.66 ± 1
CHLE	29 ± 1
Control	59.66 ± 1

The pH values were relatively stable during the experiment and no difference was seen between uncoated and coated fruits at 0, 1, 4 and 7 days of storage.

The effect of chitosan, LE and CHLE on mycelial radial growth

The results of measuring decay diameter indicated that the inhibitory effect of the CHLE on *P.expansum* was significant ($p \leq 0/05$), in compared with chitosan and LE alone at the 4, 7 and 14 days of storage. At the end of storage, the highest decay incidence were observed in controls(59.66 mm), LE(43 mm), chitosan(38 mm) and CHLE(29 mm), respectively. The results of lesion diameter measurement are presented in Table 2. Chitosan blended with vanillin was more effective compared to vanillin and chitosan alone against Botrytis cinerea [12]. Chitosan coating combined with *Origanum vulgare L* essential oil, maintained shelf life quality of table grape as well as controlled *Rhizopus stolonifer* and *Aspergillus niger* in postharvest storage [24]. The decay development of coated and uncoated fruit is shown in Fig. 2.

Edible coatings are beneficial strategies that are applied to preserve postharvest quality of fruits and vegetables. The coatings can expand the shelf life of products by retaining the moisture and aroma, modifying the atmosphere and preventing microbial growth.

Chitosan is widely used to prolong fruit and vegetable shelf life. This polysaccharide is able to form a semi permeable layer on product surface and enhance the quality of products by modifying the internal atmosphere and restricting transpiration losses [25].

Conclusions

The conclusions of our research clearly indicated that the CHLE declined loss weight and extension of firmness and total soluble solid due to retention of moisture during the storage time.. Furthermore, chitosan-liquorice edible coating inhibited *penicillium expansum* growth and reduced postharvest decay rate. In general, chitosan has more effectiveness when combined with liquorice extract. CHLE. The results of this research support the idea that CHLE coating may be as a safe alternative method to prolong shelflife and reduction postharvest losses of apple and maybe other fruits in storage time.

Fig. 2 The in vivo antifungal assay of chitosan (1), LE (2), CHLE (3) and control (4) after14 days incubation at 25°c

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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