Biocontrol of Gray Mold Disease on Strawberry Fruit by Integration of *Lactobacillus plantarum* A7 with Ajwain and Cinnamon Essential Oils

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Abstract: This study was conducted to evaluate the efficacy of the *Lactobacillus plantarum* A7 (*L. Plantarum*), ajwain and cinnamon essential oils (AO and CO, respectively) in suppressing gray mold rot in strawberry fruit. AO and CO showed over 90% inhibition of radial mycelia growth with lower concentration of the oils per plate for all tested pathogens. Combined application of *L. plantarum* with AO and CO was tested to assess the possible synergistic effects of these 3 elements on the control of tested plant pathogens. In this case both combinations of *L. plantarum* + AO and *L. plantarum* + CO inhibited the mycelia growth of the pathogens completely. Results showed that the combined treatment of strawberry fruits with *L. plantarum* + AO (50 μL) and *L. plantarum* + CO (100 μL) resulted in remarkably improved control of *Botrytis* infections, in comparison with application of *L. plantarum* or essential oils alone. Quality attributes (that is pH, acidity, vitamin C, and total soluble solid) of the strawberry fruits did not change significantly ($P < 0.01$) when combination of *Lactobacillus* and essential oils was used. To the best of our knowledge, this is the first report on the effects of combination of a *Lactobacillus* as an antagonist bacterium with essential oils to increase the shelf life of strawberry.

Keywords: ajwain, antagonist, biocontrol, cinnamon, essential oil, strawberry

Practical Application: The combination of antagonist bacteria and essential oils has emerged as a safe alternative to replace synthetic preservatives. According to these findings, the combination of *L. plantarum* A7 and the essential oils of cinnamon and ajwain can be used as preservative to increase the shelf life of fruits in postharvest stage.

Introduction

Strawberry is an especially perishable fruit, being susceptible to mechanical injuries, desiccation, decay, and physiological disorders during storage. Botrytis cinerea is known to be a major spoilage causing microorganisms in strawberry (Tournazs and Katsoudas 2005). This microorganism (gray mold rot) is ubiquitous plant pathogen causing severe damages pre and postharvest in many fruits, vegetables, and ornamental crops (Bouchra and others 2003; Xu and others 2007). Therefore, finding suitable methods for preserving the quality of strawberries during storage is important. Currently, control of plant diseases mainly depends on the use of chemical fungicides. However, the application of synthetic fungicides has led to a number of environmental and health complications. Hence, there seems to be an urgent need for alternative methods of pest control to replace agrochemical utilization. Essential oils are concentrated, hydrophobic aromatic oily liquids obtained from plant material (Bauer and others 2001). They were previously reported to have biological activities such as antifungal and antibacterial effects on fruits (Roller and Seedhar 2002; Dikbas and others 2011) and vegetables. The biological control by antagonist microorganisms is known to be one of the most promising nonfungicidal means especially for the control of the wound invading pathogens (Yu and others 2007). Lactic acid bacteria (LAB) are considered to be food grade microorganisms and generally recognized as safe by US food and drug administration. The success of LAB in preventing the growth and activity of foodborne pathogens and spoilage microorganisms in a large variety of foods may be due to their diverse range of antagonistic mechanisms. To our knowledge, the application of LAB in combination with essential oils in strawberry has not been described yet.

Among the essential oils those of cinnamon (CO) and ajwain (AO) are 2 important ones. *Cinnamomum verum*, called "true cinnamon" is Ceylon cinnamon or Sri Lanka that is native to Sri Lanka and South East Asia. There are a lot of investigations on antifungal and antibacterial effects of this plant *in vitro* (Tzortzakis 2009), whereas *in vivo* studies on food especially fruits and vegetables are rare.

Despite its unambiguous English name ajwain, *Carum copticume L.*, is one of those spices often misrepresented with the other plants like Anise (*Pimpinella anisum* L.). There are only a few studies on the antifungal and antibacterial properties of ajwain.

The objective of this study is to evaluate the potential of the combination of *Lactobacillus plantarum* A7 and essential oils of ajwain and cinnamon in controlling *Botrytis, in vitro* and *in vivo*, on strawberry.

Materials and Methods

Materials

Isolation of essential oils. Cinnamon, *C. verum*, oil was purchased from Zardband Pharmaceuticals-Medicinal Plants Production Company, Tehran, Iran (with its GC–MS profile). Ajwain seeds, *C. copticume L.*, were purchased from a local store in

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Isfahan, Iran. A total of 300 g of the seeds was hydrodistilled for approximately 5 h using a Clevenger-type apparatus. It was then dried over anhydrous sodium sulfate. The analysis of the extracted ajwain essential oil (AO) was performed using GC–MS system to identify the extracted oil components. All essential oils were stored in air-tight sealed glass vials covered with aluminum foil and kept at approximately 4 °C until use (Al-Reza and others 2010).

Microbial strains and inoculums preparation. In this study *L. plantarum* A7 as an antagonistic bacterium and *Botrytis* spp. as a plant pathogenic mold were used. *Botrytis* spp. was isolated from infected strawberry fruits. A loop full of mold mycelium was transferred from fruits to potato-dextrose agar (PDA, Merck, Germany), and the cultivated plates were incubated at 27 °C for 7 d. Among different isolated molds, *Botrytis* spp. was identified according to the cultural and morphological characteristics (Chilvers and Du Toit 2006). *Botrytis* was maintained on PDA at 4 °C, and was refreshed on PDA plates at 27 °C for 7 d before usage. The concentration of the *Botrytis* spore suspensions (10^4 spore/mL) was determined using a haemocytometer and a compound light microscope. *L. plantarum* A7 was obtained from Culture Collection of Food Biotechnology and Microbiology Laboratory, Department of Food Science and Technology, Faculty of Agriculture, Isfahan University of Technology, Isfahan, Iran. *L. plantarum* A7 was grown in De Man, Rogosa and Sharpe (MRS, Germany) broth for 15 h at 37 °C (Alegre and others 2011). Bacterial concentration was estimated using a spectrophotometer (Unico-uv-2100 spectrophotometer, New Jersey, USA) set at $\lambda = 600$ nm according to previously determined standard curves. Bacterial cells were harvested by centrifugation at 15344 × *g* for 15 min at 10 °C. The broth was decanted and the cells were suspended in sterile distilled water to obtain a suspension containing approximately 10^8 cfu/mL cells. Inoculum concentration was checked by plating appropriate dilutions on to MRS-agar for *L. Plantarum* and PDA for *Botrytis* spp.

Methods

Gas chromatography–mass spectrometry (GC–MS) analysis. The analysis of the essential oils was performed using a Agilent technology 5975C Germany GC, equipped with a HP-5MS capillary column (30 m, 0.25 mm i.d., 0.25 mm film thickness) and a mass spectrometer 5973 as its detector. The carrier gas was helium, by a flow rate of 1 mL/min. Column temperature was initially kept for 3 min at 50 °C, and then was gradually increased to 300 °C at 4 °C/min. It was then kept constant at 300 °C for 20 min. For GC–MS detection, an electron ionization system was used with ionization energy of 70 ev. Injector and detector temperatures were set at 230 and 310 °C, respectively (Tzortzakis 2009).

Identification of components. The profile of the extracted essential oils were identified by comparing their mass spectra with that of those present in the computer library or with pure components, as confirmed by comparison of their retention time.

The impact of CO and AO on pathogen development *in vitro*. For *in vitro* evaluation of antifungal activity, 2 methods, "contact phase" and "volatile phase," were used (Soylu and others 2010). In contact phase method, required amounts of essential oils were diluted in 0.5 mL, 96% ethanol, in order to make a better distribution of the essential oils in the culture media (PDA). Each vial of diluted essential oil was added to the plates containing 9.5 mL liquid PDA at 45 °C to reach the final concentrations of essential oils of 0.5, 1, 1.5, 2, 2.5, 3, and 10 μL/mL. In the volatile phase method 9.5 mL of PDA was poured in each plate. After solidification, a piece of filter paper was placed on each PDA plates and an appropriate amount of each essential oil was dropped onto the filter papers. In both methods, a 0.5 to 1 mm plug of mold mycelium from the edge of 6-day-old *Botrytis* spp. plates was located in the center of plates. Plates were used in 3 replicates for each treatment and then the inoculated media were incubated at 27 °C and mycelium growth was determined daily. Growth measurement was determined using the following formula (Lee and others 2007):

$$\mathbf{IP = dc - dt \times 100/dc}$$

where IP is inhibition percentage, *dc* is the colony diameter size in untreated plates (that is blank sample) and *dt* is the diameter size (3 replicates) in plates treated with individual essential oils at the tested concentration range. IP was reported for each essential oil after 5 d.

The nature of antifungal activity of the essential oils, that is fungistatic or fungicidal action, was determined. The inhibited fungal plugs were placed upside down on the surface of PDA plates without essential oil and the result was recorded on the 7th day of incubation at 27 °C. Fungal growth indicated fungistatic action, while the absence of growth indicated fungicidal action.

***In vitro* antagonistic effects of *L. plantarum* A7 on *Botrytis* spp.** Screening for antagonistic activity of *Lactobacillus* was carried out based on Trias and others (2008) method. *L. plantarum* was spotted on MRS agar plates (3 replicates) and incubated 24 h at 27 °C. *Botrytis* spp. spore suspension (0.5 mL) containing 10^4 spore/mL was mixed with 4.5 mL MRS soft agar (0.7% agar) and overlaid on the plate containing grown colonies of *L. plantarum*. Plates were incubated at 27 °C for 24 h. The diameter of the inhibition area was measured. Inhibition diameters (mm) were normalized for each experimental condition by dividing values to maximum value observed. The ratio obtained values were categorized into 5 levels of activity corresponding to high (1.00 to 0.90), moderate (0.89 to 0.70), medium (0.69 to 0.40), and low (0.39 to 0.20) and without significant activity (0.19 to 0.00)

***In vitro* study on combined effects of essential oils and *Lactobacillus* on mycelia growth.** Each essential oil was used with *L. plantarum* individually to investigate their combined effects on *Botrytis* spp. based on volatile phase method. Liquid culture of *L. plantarum* incubated at 37 °C for 15 h and was then added to the PDA before pouring in to petri dishes. After solidification, a piece of watman filter paper was placed on PDA plates and an appropriate amount of each essential oil was dropped to the filter paper. A 0.5 to 1 mm plug of mycelium from the edge of 6-day-old *Botrytis* plates was placed in the center of PDA plates. Plates were used in 3 replicates for each treatment and then inoculated media were incubated at 27 °C. The inhibitory effect was reported after 5 d visually and ranked in a 1 to 5 visual scale (1 = 0%, 2 = 25%, 3 = 50%, 4 = 75%, 5 = 100% growth.)

***In vivo* investigation of effects of essential oils on decay development in artificially inoculated by *Botrytis*.** After *in vitro* evaluation of antifungal properties of the essential oils, the volatile phase method was selected for the evaluation of the effect of essential oils on postharvest decay and some quality attributes of strawberry. Strawberry fruits (*Fragaria x ananassa*) were obtained from a local company and immediately transported to our laboratory (Isfahan University of Technology, Iran). Uniform strawberries by shape, size, and color were selected. These fruits were treated by 96% ethanol, rinsed with tap water and after draining in room temperature, were transferred to 1 L polystyrene containers with cap. Each fruit was wounded with a sterile puncher to make one uniform 2-mm deep by 4-mm wide wound on its equatorial

region. Conidia of *Botrytis* were recovered from a 2-week-old culture by adding 10 mL of sterile water and 0.02% tween 80 to each plate. The mycelial suspension was filtered through a piece of sterile cheese cloth. The concentration of the conidial suspension was adjusted to 10^4 spores per mL, then, each fruit was inoculated with 20 μL of the conidial suspension. A piece of watman filter paper (number 1) was placed in the center of containers and treated by the required amounts of each essential oil (50, 100, and 200 μL) a moistened filter paper (RH approximately 95%) was also located in the containers in order to maintain a high relative humidity during experiment (Figure 1). Treated fruits were stored at 15 °C. The percentage of infected fruits was recovered after 7 d of incubation. Each treatment was replicated 3 times with 8 fruits per replicate (Corato and others 2010).

***In vivo* investigation of combined effects of essential oils and *L. plantarum* A7 on decay development in artificially inoculated fruits by *Botrytis*.** Uniform strawberries by shape, size, and color were selected. These fruits were washed and drained and wounded as explained in former section. Wounded fruits were sprayed with *L. plantarum* (10^8 cfu/mL) via a medicinal syringe 10 h prior to pathogen inoculation. Then, conidia of *Botrytis* spp. were recovered from the 2-week-old cultures. Each fruit was inoculated with 20 μL of the conidial suspension. A piece of watman paper (number 1) was placed in the center of containers and treated by the required (50, 100, and 200 μL) amounts of each essential oils and also a moistened filter paper (RH approximately 95%) was located in the containers in order to maintain a high relative humidity during storage. Treated fruits were stored at 15 °C. The percentage of infected fruits was recovered after 7 d of incubation. Each treatment was replicated 3 times with 8 fruits per replicate (Corato and others 2010).

Quality analysis of strawberries

Total soluble solid. Total soluble solid (TSS) of fruit juice for each treatment was determined using Bellingham & Staley LTD (England) refractometer at 20 °C, expressed as °Brix.

Figure 1–Preparation of strawberries for *in vivo* investigation of effects of essential oils on decay development in artificially inoculated and wounded fruits.

Table 1–The chemical composition of the ajwain essential oil.

	Compounds	Retention time (min)	Area%
1	β-pinene	4.68	21.75
2	γ-terpinene	4.98	18.90
3	Myrcene	6.75	59.35

Table 2–The chemical composition of the cinnamon essential oil.

	Chemical compounds	Retention time (min)	Area%
	Monoterpene hydrocarbons		
1	α-pinene	3.87	2.73
2	β –pinene	4.66	4.24
3	γ-terpinene	4.53	2.72
4	Δ-3-carene	11.53	1.82
5	p-Cymene	4.66	4.24
	Oxygenated monoterpenes		
6	1,8-cineol	4.73	5.03
7	Linalool	5.28	6.56
8	Eugenol	7.32	8.55
9	Cinnamyl acetate	7.80	2.05
10	Cinnamylaldahyde	6.76	46.31
11	p- Methoxycinnamaldehyde	8.40	7.75
	Sesquiterpenes		
12	ß-Caryophyllene	7.73	8.00

pH. The pH of fruit juice was measured using a Jenway 3330 pH meter (Fisher Scientific UK Ltd) calibrated by pH 4 and 7 buffer solution.

Titrable acidity. Titrable acidity (TA) was determined by potentiometric titration, using fruit samples (10 g) diluted in 100 mL distilled water, and titrated with 0.1N NaOH until the formation of a pink precipitate. It was monitored using phenolphthalein as the pH indicator. The reported value ($n = 3$) was expressed in terms of citric acid percentage.

Ascorbic acid. The Iodometric method was used to measure the ascorbic acid content of the pressed fruit juice. The results were expressed as milligrams of ascorbic acid per 100 g sample.

Statistical analysis

Statistical analyses of the data were performed using SAS statistical software SAS 8.2 (TS2M0) and Factorial Experiments in

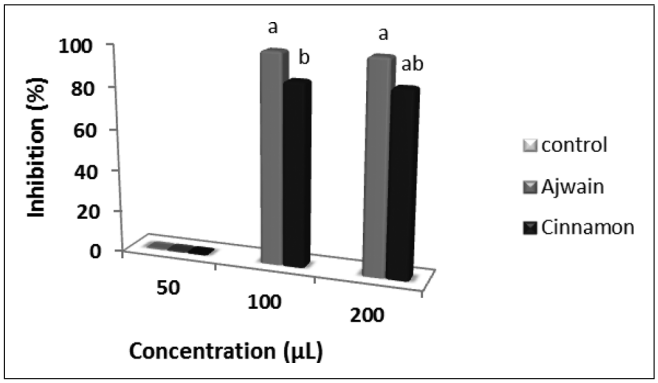

Figure 2–Effects of essential oils on protection of strawberries after inoculation with *Botrytis*.
* In this test, control samples were decayed after 3 d.
* Values followed by the same letters were not significantly different ($P < 0.01$).
* Each treatment was replicated 3 times with 8 fruits per replicate.

Completely Randomized Design. All experiments were done in replicates.

Results and Discussion

GC–MS analysis of the samples

The identified components of AO and CO are listed in Table 1 and 2, respectively, according to their elution time from the HP-5 column in GC–MS system. CO and AO were rich in monoterpenes. Their major components were: Cinnamyl aldehyde (46.31%), eugenol (8.55%), β-caryophyllene (8%), p-methoxycinnamaldehyde (7.75%), in CO and β-pinene (21.75%), myrcene (59.35%), and γ-terpinene (18.90%) in AO.

The impact of CO and AO on pathogen development *in vitro*

Antifungal activities of the plant essential oils were tested against *Botrytis* spp. Both tested essential oils showed absolute inhibition activity against *Botrytis* spp. in all tested concentrations (0.5, 1, 1.5, 2, 2.5, 3, and 10 μL/mL). To determine the fungicidic/fungistatic effect of the essential oils, after transferring the mold plugs from the essential oil treated PDA media to then on-treated media, no mycelial growth inhibition was observed on the nontreated media, indicating the fungicidial effect of essential oils against *Botrytis* spp.

Several previous studies suggested that antifungal activity of the essential oils may in part be due to the severe damage to fungal membrane and cell wall, which leads to morphological collapses and deterioration of hyphae and/or conidia (Amiri and others 2008). The amphipathicity characteristic of these components is the main reason for their interactions with biomembrane, and their antifungal activity (Asghari-Marjanlo and others 2009). In this study, the presence of some antifungal constituents such as β-pinene, myrcene, and γ-terpinene in AO and cinnamylaldehyde, eugenol, β-caryophyllene, and p-methoxycinnamaldehyde in combination with other minor constitutes in CO might have improved overall antifungal activities of essential oils.

In the present study, no notable differences was observed between the contact phase and volatile phase methods in the evaluation of the antifungal efficacy of the essential oils, whereas Asghari-Marjanlo and others (2009) reported that the volatile phase method is more effective than the contact phase method in this regard. They reported that in the contact phase method, essential oils in low concentrations lose their antifungal activity. Soylu and others (2010) observed that in the volatile phase method, the volatile inhibitory effect of essential oils on mycelia growth was greater than that in the contact phase method. They found that in this method (contact phase method); relatively higher concentrations of essential oil were required to inhibit mycelia growth. The advantages of volatile phase method for food products are that it may have less influence on the sensory characteristics of the product and its release may be regulated better (Soylu and others 2010).

***In vitro* study of antagonistic effect of *L. plantarum*A7 on *Botrytis* spp.** Screening for antagonistic activity of *L. plantarum* A7 was carried out based on Trias and others (2008) method. It was evident (results not shown) that *L. plantarum* is an efficient antagonist for inhibiting the mycelia growth of *Botrytis* (inhibition zone 40 mm). Antagonist microorganisms use different mechanisms to inhibit other bacterial growth; it seems that in this case, producing bacteriocins is a way of suppressing *Botrytis* sp. (bacteriocins were not determined in this study). Arrebola and others (2010) showed the antagonistic effect of *Bacillus amyloliquefcience* on *B. cinerea* and *Penicillium expansum* by the production of lipopeptides, which were identified as iturin A, fengycin, and surfactin.

***In vitro* investigation of combined effects of essential oils and *Lactobacillus* on mycelia growth.** Effects of AO and *L. plantarum* or CO and *L. plantarum* combined treatments on the radial mycelia growth of *Botrytis* spp. was tested *in vitro*. In this test, the lower concentration of essential oils was used. *In vitro* inhibition of mycelia growth in presence of *L. plantarum* A7 and essential oils combination was observed and scored 5 based on visual scoring 5 (data not presented). The results showed a notable increase in *L. plantarum* A7 inhibitory effect when used together with each essential oil individually. The results also indicated that inhibitory effects were more than that when essential oils were used alone. The observed effect of combined treatment of *L. plantarum* + essential oil on radial mycelia growth reduction could be due to synergistic interactions between essential oils and *L. plantarum* inhibitory action which needs further investigations. Likewise, Arrebola and others (2010) showed that the combination effects of thyme or lemongrass essential oils and *Bacillus amyloliquefaciens* PPCB004 completely inhibited *Botrytis* spp., *Penicillium* and *Rhizopus* growth.

Effect of essential oils on fungal development on strawberries *(in vivo)*. CO and AO were used to reduce the infection of strawberries after inoculation of *Botrytis* spp. The results of antifungal activity of CO and AO are shown in Figure 2. In this study, using 3 concentrations of CO (50, 100, and 200 μL) could not completely prevent fruit decay (although in case of 100 and 200 μL were effective), whereas 100 and 200 μL of AO, showed

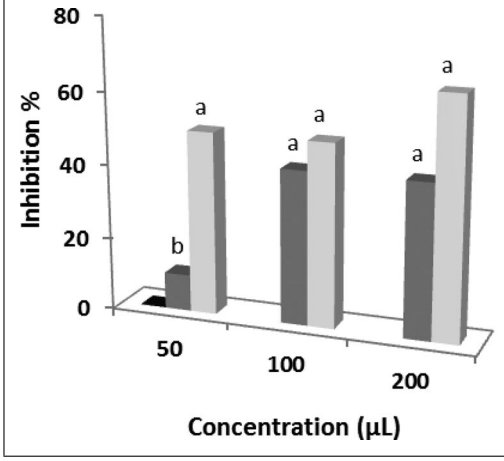

Figure 3–*In vivo* investigation of combined effects of essential oils and *Lactobacillus* on decay development in artificially inoculated and wounded fruits.
* Values followed by the same letters were not significantly different ($P < 0.01$).
* Each treatment was replicated 3 times with 8 fruits per replicate.

100% decay inhibition. The control sample fruits (without essential oil) were decayed after 3 d. However, the results of *in vivo* experiments showed that the essential oils (AO and CO, 100 and 200 μL) treated fruits maintained an acceptable quality and had low severity of decay (visual) after 3 d, whereas nontreated fruits showed full deterioration after 3 d. However, antifungal activity percentage of AO was significantly more than that observed by CO ($P < 0.01$).

Comparing inhibitory effects of essential oils on natural and inoculated fruits by *Botrytis*, It was concluded that the higher the

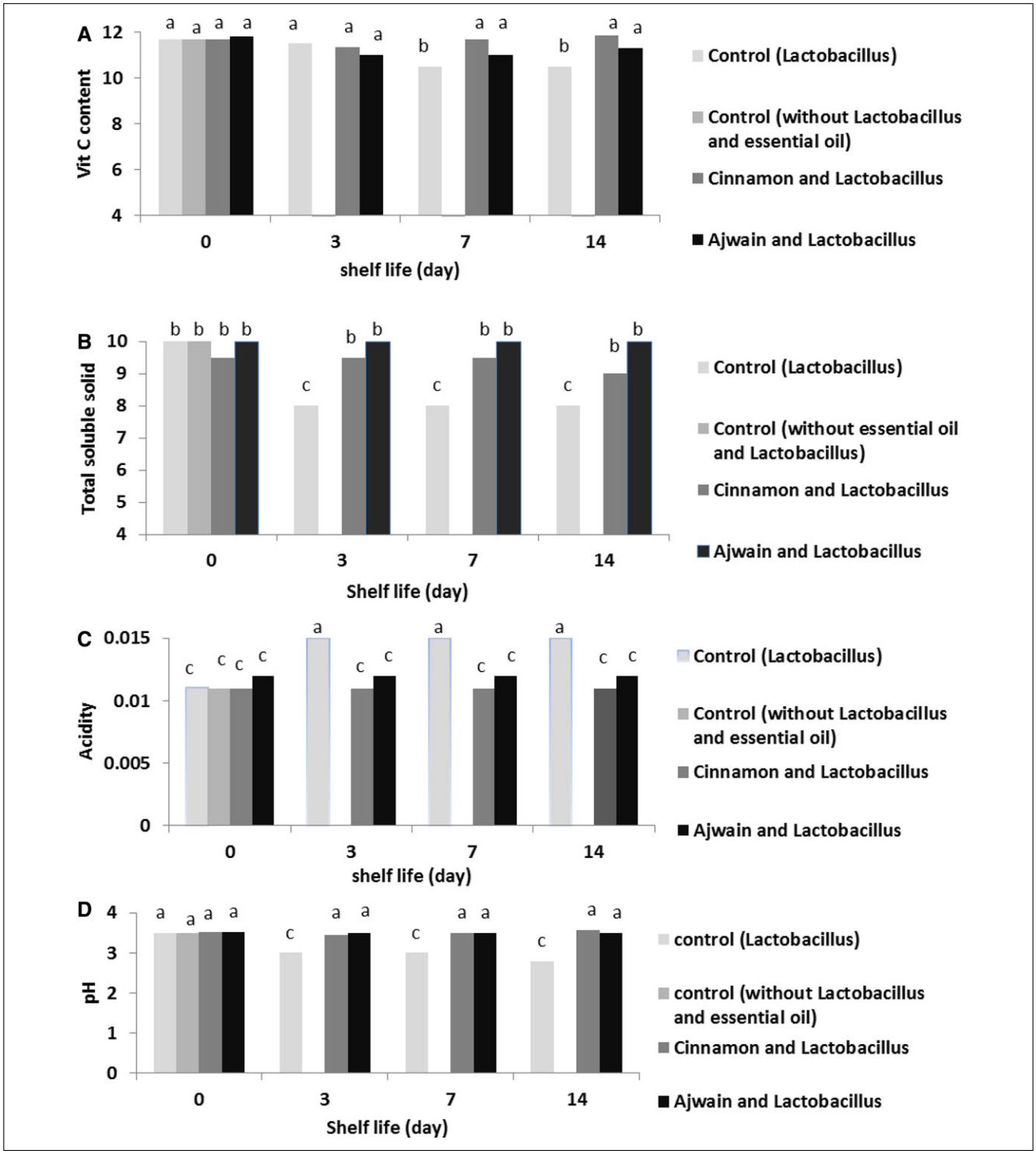

Figure 4–Combined effect of *L. plantarum* and essential oil vapors enrichment on vitamin C (A), TSS (B), acidity (C), and pH(D) of strawberries.
* The amounts of essential oils applied on the fruits were 50 μL of cinnamon and 100 μL *of* ajwain oil.
* Each treatment was replicated 3 times with 8 fruits per replicate.
* Values followed by the same letters were not significantly different ($P < 0.01$).

mold population, the more the decrease in antifungal activity of CO. It seems that the lower effects of the essential oils in protective activity compare to natural protection (without pathogen inoculation) could be due to the changes in incubation conditions, the amount of essential oil and the initial fungal load of samples. It has been reported that most antifungal volatiles and vapors reduce conidial germination subsequently, kill the fungi (Reddy and others 1998).

Fungal inhibition observed under vapor treatment, may be explained by considering the fact that hydroxyl groups in antimicrobial compounds could form hydrogen bonds with active enzymes resulting in their deactivation (Juglal and others 2002). Preventing mycelia growth by essential oil treatments could make a major contribution in limiting the spread of the pathogens by lowering the spore load in the storage atmosphere and on the fruit surface.

***In vivo* investigation of combined effects of the essential oils and *Lactobacillus* on decay development in artificially inoculated fruits by *Botrytis spp*.** Combined effects of the essential oils and *Lactobacillus* on decay development are shown in Figure 3. Our experiments showed that the combined treatment of strawberry fruits with *L. plantarum* + essential oil resulted in a remarkable increase in the percentage of disease inhibition ($P < 0.01$). *L. plantarum* + AO treatment showed a higher percentage of disease inhibition ($P < 0.01$) and severity reduction than that shown by *L. plantarum* + CO in concentration 50 μL on the fruit subjected to artificial inoculation with *Botrytis* spp. This could be due to the higher antifungal effect of AO against *Botrytis* spp. on the fruit surface. However, the combined use of *L. plantarum* and CO could increase the percentage of diseases inhibition when 100 or 200 μL of CO is used. Only a few authors studied the combined effects of antagonist microorganisms and inhibitory compounds to control the postharvest diseases of fruits. Arrebola and others (2010) demonstrated that by combining lemongrass oil with antagonist *Bacillus amyloliquefaciens* PPCB004, the fruit could be protected from the incidence of postharvest diseases. Yu and others (2007) assumed that the salicylic acid might be regarded as a secondary defense line in combination with *Cryptococcus lurentii* by induction of the fruit natural resistance.

It seems that the combined treatment of *L. plantarum* and the essential oils, which integrated the dual biological activity of biocontrol *L. plantarum* and elicitor, was more effective means of minimizing the postharvest diseases of strawberry fruit. It can be assumed that *Lactobacillus* competes with pathogens for nutrients (minerals, trace elements, and peptides) and produces bacteriocins used by *Lactobacillus* to introduce antagonistic activity. Torres and others (2007) showed, the synergistic effects of combining 2 or more different nonfungicidal postharvest treatments and increase in their efficiency in reducing the decay development in citrus.

The combined effect of *L. plantarum* and the essential oils on the quality (vitamin C, TSS, acidity, and pH) of strawberries. The combined effects of *L. plantarum* and the essential oils on some characteristics of strawberries including vitamin C (A), TSS (B), acidity (C), and pH (D) are shown in Figure 4. Since all the control samples (without *L. plantarum* and the essential oils) were fully spoiled after 3 d, data are only shown for day one in all measurements. Vitamin C, pH, and TSS of control samples in the case of using *L. plantarum* alone were decreased significantly in comparison to day 1, whereas all quality attributes did not change when combination of *Lactobacillus* and the essential oils were used. Likewise, in Arrebole and others (2010) study; combination of *Bacillus amyloliqefaciens* + Lemongrass + modified atmosphere packaging (MAP) showed absence of off-flavor, retaining overall appearance and increasing overall acceptance at market conditions after cold storage at 4 °C for 14 d. When using essential oils alone, oil vapuor treatment decreased the vitamin C content of strawberries, as compared to day one. This reduction was significant ($P < 0.01$) on day 14. The observed reduction in the amount of vitamin C could be due to the changes in the experimental conditions, including unintentional growth of mold, changes in the storage temperature and moisture, and other factors that can deteriorate the vitamin C. It seems that vitamin C is destroyed probably due to the cell wall break down by fungal infection during time progression. Titrable acidity and pH of the treated strawberries were not changed during vapor exposure at the same time (data not present). Tzortzakis (2007) showed no significant effect on total acidity of tomato and strawberry after exposure to eucalyptus and cinnamon volatile oils. In Asghari-Marjanlo and others (2009) study, only a significant pH change was reported among the treatments at 12 and 15 d and no significant pH change was observed in the treated strawberry fruits, as compared to the controls and at early determination times.

In the case of TSS evaluation, vapor resulted in a significant ($P < 0.01$) increase in TSS during exposure to the essential oils. This result was in agreement with Tzortzakis (2007) study that showed cinnamon and eucalyptus vapor had no significant effect on TSS on tomato but increased TSS level in strawberry.

In contrast, no significant TSS change was observed in treated fruits, compared to that in the control samples at all determination times in reports of Asghari-Marjanlo and others (2009).

Conclusion

The present study demonstrated the notable potential of using *L. plantarum* A7 in combination with AO and CO. The lower amount of the essential oils (50 μL for ajwain and 100 μL for cinnamon) was used in combination with *Lactobacillus* in order to reduce an unpleasant fruit odor and taste resulting from the use of essential oil.

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