Food Control 28 (2012) 157–162

Contents lists available at SciVerse ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Cassia oil for controlling plant and human pathogens on fresh strawberries

Mohamed M. El-Mogy [a,*], Beatrix W. Alsanius [b]

[a] Vegetable Crops Department, Faculty of Agriculture, Cairo University, Giza 12613, Egypt
[b] Swedish University of Agriculture Science, Department of Horticulture, Microbial Horticulture Laboratory, P.O. Box 103, SE-230 53 Alnarp, Sweden

ARTICLE INFO

Article history:
Received 21 November 2011
Received in revised form
19 April 2012
Accepted 28 April 2012

Keywords:
Escherichia coli
Essential oils
Grey mould
Postharvest
Quality
Strawberry

ABSTRACT

The inhibitory effects of cassia oil on the human pathogen *Escherichia coli* serotype O157:H7 and the plant pathogen *Botrytis cinerea* were tested *in vitro* at different concentrations (200–800 ppm). Cassia oil exhibited antibacterial and antifungal activity against both pathogens. Cassia oil at 400–800 ppm inhibited the growth of *E. coli* O157:H7 *in vitro* and on the surface of treated strawberries. Cassia oil also completely inhibited the growth of *B. cinerea* at 400–800 ppm. Spore germination and germ tube elongation of the pathogens in potato dextrose broth were strongly inhibited in the presence of 100 ppm cassia oil. Cassia oil at all concentrations reduced the percentage of decayed strawberries. Experiments on reducing the development of natural decay in strawberries gave similar results. None of the quality parameters tested (colour, total soluble solids, pH, total acidity and ascorbic acid) was affected by cassia oil treatment. Storage experiments on strawberry showed that the percentage weight loss was reduced by cassia oil treatment. Hence, cassia oil could be an alternative to synthetic chemicals for controlling human and plant pathogens on fruits such as strawberries during postharvest and storage.

1. Introduction

Postharvest decay is responsible for major losses of horticultural crops after harvest and during shipment and storage. Strawberries (*Fragaria x ananassa*) are particularly perishable fruit, being susceptible to mechanical injury, decay and physiological disorders during storage. *Botrytis cinerea* is the causal agent of grey mould, which can attack strawberry leaves, stems, flowers and fruits. This disease is currently primarily controlled by application of synthetic fungicides (Norman, 1988). However, there is increasing concern about the human health and environmental contamination risks associated with fungicide residues. In addition, the extensive use of these chemicals in commercial packing houses has led to the proliferation of resistant strains of some pathogens (Palou, Usall, Muñoz, Smilanick, & Viñas, 2002). These problems have encouraged researchers to explore alternatives to synthetic fungicides for control of postharvest diseases of fruits, including grey mould decay of strawberries.

Food safety is an increasingly important public health issue. Apart from quality concerns related to plant disease, dispersal of enteric human pathogens (i.e. verotoxin producing strains of *Escherichia coli*, *Salmonella* spp., *Campylobacter* spp., *Yersinia enterocolitica*, Norovirus) via foods consumed fresh or after minimal preparation has become an important global food safety issue. *E. coli* serotype O157:H7 is a bacterium that can cause severe food-borne disease and a number of outbreaks have been reported in many countries around the world (Sakagami et al., 2001). Studies on the control of *E. coli* O157:H7 have largely focused on the use of chemicals preservatives.

Essential oils from various plant species have been reported to exert inhibitory effects on many fungal diseases in various horticultural crops by preventing fungal development. For example, Feng and Zheng (2007) reported that cassia oil at 500 ppm completely inhibited growth of *Alternaria alternaria* on tomatoes. Fungal spore production in tomatoes can be inhibited by cinnamon oil at 25 ppm and fungal growth inhibited at 500 ppm (Tzortzakis, 2009). Antibacterial effects of essential oils have also been reported in various studies. For example, carvacrol and thymol are reported to have desired antimicrobial effects on *E. coli* through their ability to permeabilise and depolarise the cytoplasmic membrane (Xu, Zhou, Ji, Pei, & Xu, 2008). There are no previous reports on the effects of cassia oil on *B. cinerea* and *E. coli* O157:H7, so the aim of the present study was to evaluate the effect of different cassia oil concentrations on the growth of these two pathogens on whole strawberries *in vitro* and *in vivo*. The effects of cassia oil application on some quality parameters of strawberry were also evaluated.

* Corresponding author. Tel.: +20 177777390; fax: +20 235717355.
E-mail address: elmogymmm75@yahoo.com (M.M. El-Mogy).

0956-7135/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodcont.2012.04.036

2. Materials and methods

2.1. Inoculum preparation

B. cinerea was obtained from the Geisenheim Research Center (Phytomedicine Section), Germany and cultured on potato dextrose agar (PDA) media (potato starch 4.0 g, dextrose 20.0 g, agar 15.0 g, Difco, Dickinson and Co.) at 30 °C for 7 days. The gfp-tagged mutant of *E. coli* O157:H7, non-pathogenic strain (verotox-1 and -2 negative), obtained from the Swedish Institute for Contagious Disease Control (Smittskyddsinstitutet, Solna Sweden, registry no. E81186) was pre-cultured from cryo cultures (−80 °C) on LB agar + ampicillin (100 μg/ml). Colonies were picked after 18 h for inoculum preparation, in which they were centrifuged (3000 rpm) for 20 min at 4 °C after cultured overnight. Autoclaved salt solution (NaCl 0.85%) was added to the centrifuged pellet to obtain the desired density.

2.2. Essential oil

Organic essential oil (not containing synthetic chemicals and/or non-natural components) of cassia (W52, 120-5-K; d: 1.052 g/cm^3) was purchased from Aldrich-sigma (Laborchemikalien GmbH).

2.3. In vitro antibacterial assay

A microtitre method was used to test the antibacterial effect of cassia oil on growth of *E. coli* O157:H7. Petri dishes containing autoclaved LB media were inoculated with a small amount of frozen stock of *E. coli* O157:H7 cells (stored in 50% glycerol at 80 °C) and incubated at 37 °C. After 18 h, an overnight culture was prepared by inoculating 10 mL of LB broth with a single colony of *E. coli* O157:H7 and incubating at 37 °C with shaking (200 rpm). The overnight culture was centrifuged (3000 rpm) for 20 min at 4 °C. Autoclaved hypertonic NaCl solution (0.85%) was added to the pellet and adjusted to an optical density of $OD_{620} = 0.100$ (Expert 96 Model, ASYS-Hitech) at 620 nm. Double-strength LB broth was autoclaved and mixed with cassia oil to obtain final concentrations of 200, 400, 600 and 800 ppm. Cassia oil was dissolved in 0.5% ethanol (Slolveco, Sweden) before addition to the LB broth. Ethanol without cassia oil was added to make a positive control and LB broth without ethanol or cassia oil served as a negative control. Aliquots of 75 μl of *E. coli* O157:H7 suspension and 75 μl of double strength LB broth with cassia oil were mixed in sterilised 96-well microtitre plates (Sarstedt-AG&Co., Nurnbrecht, Germany). For each concentration, three replicate wells were used. The microtitre plates were covered by parafilm M® to avoid evaporation and incubated at 37 °C with shaking (200 rpm). Every 60 min from 0 to 12 h and after 24 h, the optical density at 620 nm (OD_{620}) was recorded in order to determine cell growth. The experiment was repeated.

2.4. Bacteriostat/bactericide experiment

To determine the bacteriostatic or bactericidal effects of cassia oil on *E. coli* O157:H7, cassia oil concentrations inhibitory to growth in the antibacterial assay experiment were used, employing the same microtitre procedure as mentioned above. After 0, 1, 2, 4, 8 and 16 h of incubation at 37 °C, 1 μl from the wells of each concentration were mixed with 50 μl salt buffer (NaCl, 0.85%) and spread on duplicate agar plates. After 18 h of incubation at 37 °C, the colonies were counted.

2.5. Cassia oil for reducing E. coli O157:H7 on strawberry surface

2.5.1. Bacterial incubation and essential oil treatment

In an experiment to evaluate the effect of cassia oil on the population of *E. coli* O157:H7 inhabiting the surface of strawberry (*Fragaria x ananassa*), ripe fruits of cv. Evy II were harvested from the greenhouse on the day before use and stored at 10 °C until the next day. The strawberries (2 fruits per replicate and three replicates per treatment) were inoculated with *E. coli* O157:H7 by submerging them in the bacterial suspension (10^8 CFU/ml) for 10 s. The fruits were then removed and allowed to dry for 30 min at room temperature (25 °C). This treatment yielded a fruit inoculation level of 5.42 log CFU/g fresh weight. The inoculated strawberries were immersed in a solution of cassia oil at the relevant concentration for 1 min and air-dried for 60 min under sterile conditions.

2.5.2. Determination of bacterial populations

The treated strawberries were washed in 20 ml of salt buffer (NaCl 0.85%) for 1 min in sterilized plastic bag with shaking to be sure that all attached bacteria were released. Serial dilutions were then made with the same salt buffer. Subsequently, 90 μl of the solution was spread on agar plates. The plates were then incubated at 37 °C for 18 h. Plates containing 30 to 300 colonies were counted under UV light.

2.6. In vitro antifungal assay

2.6.1. In vitro contact assay

Autoclaved and cooled potato dextrose agar (PDA) was mixed with cassia oil at a final concentration of 0, 200, 400, 600 and 800 ppm and aliquots of 15 ml were poured onto each plate. Plugs (1.3 cm) of mycelial material taken from the growing edge of a 7-day-old fungal culture were placed in the centre of each Petri dish. The plates were then incubated for 7 days at 25 °C. Fungal growth was recorded after 2, 4 and 6 days. Growth inhibition was calculated as the percentage inhibition of radial growth relative to the control. All tests were performed in triplicate and the values were expressed in centimetres. The experiment was repeated.

2.6.2. Transfer experiment

To determine the fungistatic and fungicidal effects of cassia oil, plugs showing growth inhibition were transferred to fresh PDA to assess their viability after 0, 1, 3, 5 and 7 days for three days at 29 °C. The residual fungal growth was monitored by measuring the radial growth of fungal colonies.

2.6.3. Spore germination assay

Two methods were used to assay the antifungal effect of cassia oil on spore germination. The first method used potato dextrose broth (PDB). The spore suspensions were prepared by inoculating petri dishes with a disc (1.3 cm) of 7-day-old fungal cultures and incubating at 25 °C. After 14 days, 20 ml sterilised water was added to each plate, and the surface was scraped gently with a sterile bar to release the spores. The resulting suspension was filtered through two layers of cheesecloth to remove any mycelial fragments and diluted with sterile water to the previous concentration. Cassia oil was added to a 10 ml plastic tube containing 5 ml potato dextrose broth (PDB) to obtain final concentrations 0, 20, 40, 60, 80 and 100 ppm. Aliquots (100 μl) of spore suspensions (2.1×10^7 spores ml^{-1}) of *B. cinerea* were added to each tube. After 15 h of incubation at 25 °C on a rotary shaker (200 rpm), at least 100 spores per replicate were observed microscopically (Nikon, DS-Fi1, Instrument AB, Japan) to determine germination rate and germ tube length. Germ tube length was expressed in micrometres and spore germination as a percentage. The experiment was repeated.

2.7. In vivo application of cassia oil to control postharvest fungal decay of strawberries and its effects on quality

2.7.1. Effects of cassia oil on naturally infected, unwounded strawberries

Strawberries were purchased from a local store in Scania, Sweden. Fruits free from injury and infection were used for the

experiment. The cassia oil concentrations were prepared by dissolving the requisite amounts in 50 ml 0.05% Tween-20 and then mixing with 950 ml sterile distilled water. The control sets were prepared similarly using equal amounts of sterilised water in place of the essential oil. The strawberries were immersed in the relevant solution for 1 min at room temperature (25 °C) and air-dried. Strawberries immersed in sterile distilled water for 1 min and air-dried served as the control. Treated strawberries were stored at 2 °C and 85% relative humidity for 10 days. The percentage of infected fruits and quality parameters were recorded before and after storage. Each treatment was carried out in triplicate, with 10 strawberries per replicate, and the entire experiment was repeated.

2.7.2. Effects of cassia oil on artificially inoculated strawberries

Fresh, fully coloured strawberries were immersed in a solution of 1% sodium hypochlorite for 2 min, rinsed with distilled water, and dried at room temperature (20–25 °C) for 3 h. A uniform 2-mm deep and 2-mm wide wound was made at the equator of each fruit using the tip of a sterile dissecting needle. A 30 μl portion of 200, 400, 600 or 800 ppm cassia oil or sterilised distilled water (control) was pipetted into each wound site. One hour later, 15 μL of a suspension containing 5×10^5 spores/ml *B. cinerea* was inoculated into each wound. After air-drying the strawberries were placed in plastic boxes (250 g) and stored at 2 °C for 10 days. The percentage of infected fruits was recorded after storage. There were three replicate trials, with 10 strawberries per treatment, with complete randomisation in each test. The test was repeated.

2.7.3. Quality evaluation

Weight loss, colour, pH, soluble solids content (SSC), total acidity (TA) and ascorbic acid (AA) were measured to check the quality of strawberries after cassia oil treatment in the natural infection experiment. A Minolta Chroma Meter (model CR-200, Japan), calibrated with a white plate before use, was used to evaluate colour. The $L^*a^*b^*$ colour space was used. Juice samples were obtained by processing the strawberries from each replicate in a blender. The SSC of the juice was measured with a digital refractometer (RFM 80, Precision Instruments, England) and expressed as a percentage. An automatic titrator (Titroline easy, Schott Instruments) equipped with pH meter was used to measure pH and TA. A 5 g juice sample per replicate was diluted with 20 ml distilled water and titrated with 0.1 N NaOH to pH 8.1. The TA was then calculated as percentage citric acid (predominant acid in strawberries). The AA content was determined on homogenised strawberries prepared with a Waring Blender 8011G (Model 91-358 50-60 HZ), Dynamics Corporation (New Hartford, America). Three replicate samples were prepared for each sampling date. A 5 g portion of the homogenate was extracted with 25 ml 1.5% meta-phosphoric acid for each replicate sample and stored at −80 °C until analysis. Samples were then thawed and centrifuged at 12,500×*g* for 12 min. The amount of AA was determined after a reduction treatment by dithiothreitol (DTT) (Esteve, Farré, Frigola, & Garcia-Cantabella, 1997). Samples were run on a 6000 HPLC system (Merck-Hitachi, Burladingen, Germany) with a UV detector at 248 nm (binary gradient; solvent A 20 mM K2HPO4: methanol (96:4 with pH adjusted to 2.3 using concentrated H3PO4), solvent B 100% methanol). The flow rate was 1 ml min^{-1} and the injection volume 10 μL. Separation was performed with a Phenomenex synergy column (polar RP 250 × 4.6 mm, particle diameter 4 μm) and quantification was carried out by comparison with an external standard, L (+) ascorbic acid (VWR International, Leuven, Belgium). A mobile phase gradient was used as follows: 0–4 min: 0% eluent B, 4–7.5 min: 0–80% B, 7.5–8 min: 80-0% B, and 8–18 min: 0% B.

3. Statistical analyses

All experiments were repeated twice. Statistical analyses of the pooled data from the two runs were performed with Minitab 15 statistical software.

4. Results

4.1. In vitro antibacterial assay

The inhibitory effect of cassia oil at 0, 200, 400, 600 and 800 ppm concentrations on growth of *E. coli* O157:H7 is shown in Fig. 1. Cassia oil at all concentrations gave inhibitory effects on *E. coli* O157:H7 growth compared with the control. However, there was a rapid increase in OD_{620} at 200 ppm after 3 h of incubation and a slight increase at 400, 600 and 800 ppm with increasing time of incubation. No significant difference in OD_{620} was observed at 400 and 600 ppm during the entire incubation time. The most effective concentration was 800 ppm.

4.2. Bacteriostat/bactericide experiment

The experiment to distinguish between bacteriostatic and bactericidal effects of cassia oil on *E. coli* O157:H7 indicated that the oil acted as a bacteriostat at all concentrations at optimum growth temperature (37 °C). The cells grew when spread on normal agar media without any essential oils after 24 h of incubation at 37 °C (data not shown).

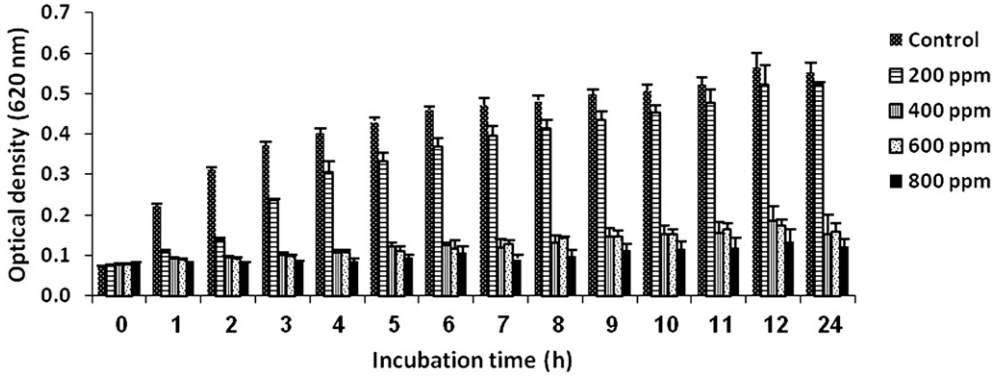

Fig. 1. Effect of cassia oil at various concentrations in inhibiting growth of *E. coli* O157:H7, expressed as optical density, during incubation for 24 h at 37 °C. Bars represent standard errors.

4.3. Cassia oil for reducing E. coli O157:H7 on strawberry surface

The initial inoculation level of *E. coli* O157:H7 on the surface of strawberries was 5.42 log CFU/ml. The reduction in the bacterial community after treatment of inoculated strawberries with different cassia oil concentrations is presented in Fig. 2. Treatment of the inoculated fruit with cassia oil at concentrations of 0 (water), 200, 400, 600 and 800 ppm resulted in an approximately 12, 23, 25, 28 and 48% reduction, respectively, in the *E. coli* O157:H7 population on the surface of the strawberries.

4.4. In vitro antifungal assay

4.4.1. In vitro contact assay

As shown in Table 1, cassia oil at 600 or 800 ppm completely inhibited mycelial growth of *B. cinerea* after 2, 4 and 6 days of incubation at 25 °C. With increasing incubation time, the inhibition effect at 200 or 400 ppm was reduced. There was a significant positive correlation ($r \geq 0.9$) between inhibition ratio and cassia oil concentration.

4.4.2. Transfer experiment

There was no effect of cassia oil at a concentration of 200 ppm on inhibition of mycelial growth of *B. cinerea* during the incubation period (data not shown). At 400 ppm, cassia oil acted as a fungistatic agent after all periods of exposure (Fig. 3a). Moreover, inhibition rate increased with increasing exposure time. The same trend was observed at 600 ppm (Fig. 3b), where cassia oil acted as a fungistatic agent during the first 3 days of incubation. The greatest inhibition in growth of *B. cinerea* was observed after 5 and 7 days of exposure.

Cassia oil at 600 ppm was more effective than 400 ppm during the first 3 days of incubation (Fig. 3). Cassia oil at 800 ppm acted as a fungicidal agent after 1, 3, 5 or 7 days of exposure (data not shown). Thus the fungistatic or fungicidal effects of cassia oil were mainly determined by the concentration and partly by the exposure time.

4.4.3. Spore germination assay

The inhibitory effect of cassia oil at 0, 20, 40, 60, 80 and 100 ppm on spore germination and germ tube length is presented in Table 2. All concentrations of cassia oil significantly inhibited spore germination and germ tube length of *B. cinerea*. Germination was completely inhibited at 100 ppm after 15 h of incubation at 25 °C. Germ tube length was 2.03 μm at 80 ppm, compared with 50.38 μm in the untreated control (Table 2).

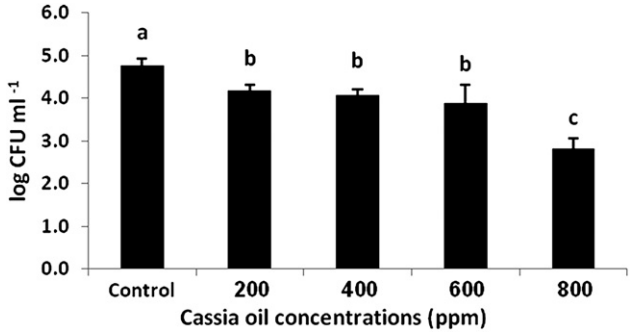

Fig. 2. Effect of cassia oil at various concentrations in reducing *E. coli* O157:H7 populations (log CFU/ml) on the surface of strawberries. Bars represent standard errors.

Table 1
Effect of cassia oil at various concentrations and periods of incubation on mycelial growth of *B. cinerea*.

Inhibition (%)						
	Control	200 ppm	400 ppm	600 ppm	800 ppm	r
2 days	0.00 d[a]	37.5 c	75.0 b	100 a	100 a	0.95
4 days	0.00 d	26.3 c	44.7 b	100 a	100 a	0.96
6 days	0.00 d	13.2 c	21.1 b	100 a	100 a	0.92

[a] Treatments followed by different letters are statistically different following the Tukey test ($p < 0.05$).

4.5. In vivo application of cassia oil to control postharvest fungal decay of strawberries and its effects on quality

4.5.1. Effects of cassia oil on naturally infected, unwounded strawberries

All concentrations of cassia oil decreased percentage decay in naturally infected, unwounded strawberries stored at 2 °C and 90% relative humidity for 10 days compared with the control (Fig. 4). Treatment with 800 ppm cassia oil reduced the proportion of decayed strawberries to 19%, while in the control the corresponding proportion was 48%.

4.5.2. Effects of cassia oil on artificially inoculated strawberries

Concentrations of cassia oil at 600 and 800 ppm decreased significantly the percentage decay in artificially inoculated strawberries stored at 2 °C and 90% relative humidity for 10 days compared with the control (Fig. 5).

4.5.3. Quality evaluation

Weight loss of strawberries was significantly decreased by all cassia oil concentrations compared with the control (Table 3). However, there was no difference between the concentrations. The red colour of skin (a/b), skin luminosity (L^*), TSS, pH, TA and AA were not affected by cassia oil treatment at any concentration after 10 days of storage at 2 °C.

5. Discussion

Microbial contamination either with human pathogens or plant pathogens poses a substantial risk to producers and consumers.

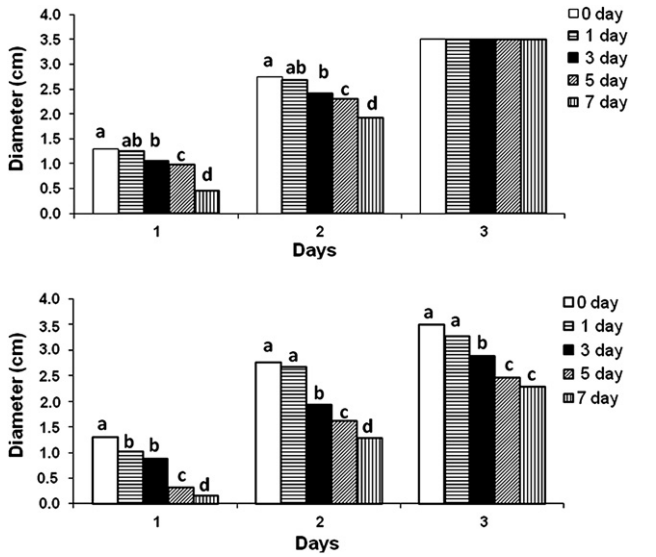

Fig. 3. Effect of cassia oil at a concentration of 400 ppm (A) and 600 ppm (B) and 0–7 days of exposure in inhibiting mycelial growth of *B. cinerea* after 1, 2 and 3 days of incubation at 37 °C.

Table 2
Effect of cassia oil at various concentrations on spore germination (%) and germ tube length (μm) of*B. cinerea* in PDB medium.

Treatment (ppm)	Spore germination %	Germ tube length (μm)
0	100.00 a[a]	50.38 a
20	97.63 a	17.44 b
40	46.25 b	7.94 c
60	11.13 c	2.14 d
80	2.13 d	2.03 d
100	0.00	0.00

[a] Treatments followed by different letters are statistically different following the Tukey test ($p < 0.05$).

This is the first study to consider both aspects in connection with strawberries. Strawberries are an important crop for the fresh market and a high-value alternative for off-season production and export. Avoidance due to low consumer quality imposes an economic penalty. With increasing fungal diseases which can infect and damage some perishable berry crops such as strawberries and concerns about the human health and environmental contamination risks associated with extensive use of chemical fungicides, there has been increased interest in finding alternatives to the use of synthetic fungicides for postharvest disease control. Previous studies indicate that *E. coli* O157:H7 can survive on fresh strawberry surfaces (Knudsen, Yamamoto, & Harris, 2001; Yu, Newman, Archbold, & Hamilton-Kemp, 2001). Contamination by *E. coli* O157:H7 can occur during production, harvest, packing, transportation or marketing. However, strawberries are not washed during those processes due to their high susceptibility to fungal infection. As a result, contaminated strawberries could pose a risk of infection to the consumer. One of the most promising natural antifungal and antibacterial agents is essential oils. Of the five essential oils evaluated here in terms of inhibiting the growth of *B. cinerea* and *E. coli* O157:H7 *in vivo* and *in vitro*, the most active oil was cassia oil.

The inhibitory effect of cassia oil at concentrations of 400, 600 and 800 ppm against *E. coli* O157:H7 (Fig. 1) can be attributed to cinnamaldehyde (3-phenyl-2-propenal), which is considered the main compound (65%) in cassia oil. The mode of action of cinnamaldehyde is still unclear. One previous study showed that cinnamaldehyde inhibited the growth of *E. coli* O157:H7, but did not destroy the outer membrane or deplete the intracellular ATP pool (Helander et al., 1998). The mechanism of action of cinnamaldehyde could involve binding to proteins, which is reported to prevent the action of amino acid decarboxylases in *Enterobacter aerogenes* (Wendakoon & Sakaguchi, 1993). The mechanism of action of most essential oils is attributed to their hydrophobicity, which enables them to partition the lipids of the microbial cell membrane, disturbing the structures and rendering them more permeable

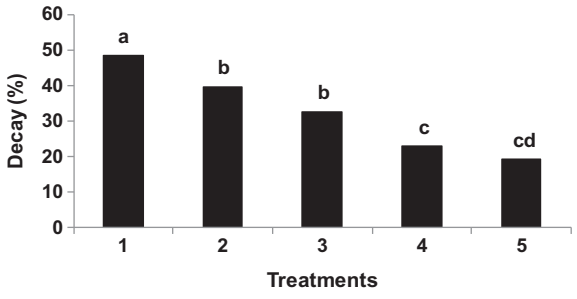

Fig. 4. Effects of cassia oil on naturally infected strawberries stored at 2 °C for 10 days: (1) Control, (2) 200 ppm, (3) 400 ppm, (4) 600 ppm and (5) 800 ppm. Treatments followed by different letters are statistically different following the Tukey test ($p < 0.05$).

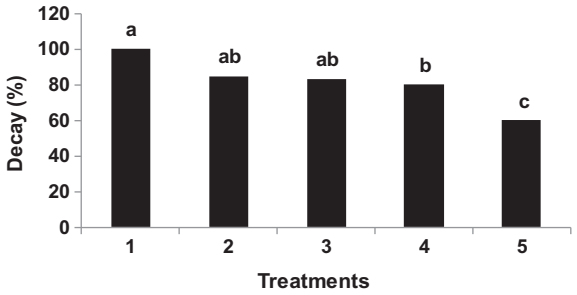

Fig. 5. Effects of cassia oil on artificially inoculated strawberries stored at 2 °C for 10 days: (1) Control, (2) 200 ppm, (3) 400 ppm, (4) 600 ppm and (5) 800 ppm. Treatments followed by different letters are statistically different following the Tukey test ($p < 0.05$).

(Bagamboula, Uyttendaele, & Debevere, 2004; Lambert, Skandamis, Coote, & Nychas, 2001).

Most of the studies to date have tested the inhibitory effect of essential oils on microbial growth *in vitro* and few have studied the *in vivo* effects (Newman, Archbold, & Hamilton-Kemp, 2001). In the present study on the *in vivo* efficacy of cassia oil, the population of *E. coli* O157:H7 on the surface of strawberries was significantly reduced by cassia oil treatment (Fig. 2).

Cassia oil also completely inhibited *in vitro* growth of *B. cinerea* in this study. Most essential oils have been reported to inhibit postharvest fungal growth *in vitro*. Feng and Zheng (2007) found that cassia oil exhibited antifungal activity against *Alternaria alternata* and completely inhibited its growth at 300–500 ppm. Cassia oil acts as a fungistat at low cassia oil concentrations and long-term exposure, or as fungicide at high concentrations (Fig. 3a,b). Similar effects on *A. alternata* have been reported by Feng and Zheng (2007).

The percentage decay of artificially *B. cinerea*-inoculated and unwounded fruit was significantly reduced by cassia oil application (Figs. 4and 5). The inhibitory effects of cassia oil concentrations were more effective *in vitro* than *in vivo*. This could be due to interactions between phenolic compounds and the food matrix (Nychas & Tassou, 2000).

Treatment with cassia oil did not affect the quality parameters tested and in fact weight loss of strawberries was decreased by all concentrations (Table 3). Loss of weight in vegetables during storage is caused by water exchange between the internal and external atmosphere, the transpiration rate being accelerated by cellular breakdown (Woods, 1990). The higher fungal spoilage of control strawberries would lead to tissue disruption and could be responsible for the higher weight loss reported in this study. Likewise, Martinez-Romero et al. (2008) reported that colour, firmness, TSS and TA were similar in lettuce treated with mint oil and the untreated control. In addition, they noted higher weight loss in control lettuce compared with treated lettuce due to higher

Table 3
Effect of different cassia oil concentrations on weight loss, L^*, a/b, TSS, pH, TA and AA of strawberries after 10 days of storage at 2 °C.

Treatments (ppm)	Weight loss (%)	L^*	a/b	TSS (%)	pH	TA (%)	AA (mg/100 g fw)
0	14.68 a[a]	33.74[ns]	2.76[ns]	8.5[ns]	3.55[ns]	0.78[ns]	36.21[ns]
200	11.67 b	33.79	2.74	8.5	3.64	0.82	36.24
400	11.43 b	33.72	2.98	8.5	3.59	0.83	36.86
600	11.92 b	33.17	3.15	8.5	3.59	0.82	36.49
800	11.79 b	33.11	2.79	8.4	3.56	0.83	36.81

ns: Non significant.
[a] Treatments followed by different letters are statistically different following the Tukey test ($p < 0.05$).

respiration rate in the former leading to accelerated metabolism and tissue senescence. Another study found that weight loss was significantly reduced by addition of eugenol, thymol or menthol oil to sweet cherries (Serano, Martinez-Romero, Castillo, Guillén & Valero, 2005). However, the mechanism by which these essential oils lead to a reduction in weight loss is still unclear.

Application of essential oils for reduction of grey mould and risk of dispersal of verotoxin producing *E. coli* is of course connected with increased costs. Essential oil treatment can preferentially be treated in advanced strawberry washing line with a final washing bath; these are available from different manufacturers. Per ton of strawberry, administration of cassia oil at 200 ppm, 400 ppm, 600 ppm and 800 ppm leads to additional costs of 1.53 Euro, 2,61 Euro, 3.69 Euro and 4.76 Euro, respectively. Considering the economic consequences caused by outbreaks of food-borne pathogens related to strawberries or parallel commodities, these costs ought to be regarded as marginal.

6. Conclusion

In conclusion, our results showed that the cassia oil had highly positive effect against grey mould caused by *B. cinerea* in strawberry fruit. Also, cassia oil exhibited antibacterial activity against *E. coli* O157:H7. The percentage weight loss of strawberry fruits was reduced by cassia oil treatment. In addition, none of the quality parameters tested was affected by cassia oil treatment. Therefore, cassia oil could be an alternative to non-chemical control of *B. cinerea* and *E. coli* O157:H7.

Acknowledgements

The Swedish Institute, Stockholm, is acknowledged for a guest research grant to the first author, supporting his guest research period in Sweden. The present study was performed within the framework of Tvärlivs-project 2011-257 and the postgraduate school μHORT (www.phd-microhort.se), both funded by the Swedish research council Formas, which is gratefully acknowledged.

References

Bagamboula, C. F., Uyttendaele, M., & Debevere, J. (2004). Inhibitory effect of thyme and basil essential oils, carvacrol, thymol, estragol, linalool and p-cymene towards *Shigella sonnei* and *S. flexneri*. *Food Microbiology, 21*(1), 33–42.

Esteve, M. J., Farré, R., Frigola, A., & Garcia-Cantabella, J. M. (1997). Determination of ascorbic and dehydroascorbic acids in blood plasma and serum by liquid chromatography. *Journal of Chromatography B: Biomedical Sciences and Applications, 688*(2), 345–349.

Feng, W., & Zheng, X. (2007). Essential oils to control *Alternaria alternata* in vitro and in vivo. *Food Control, 18*(9), 1126–1130.

Helander, I. M., Alakomi, H.-L., Latva-Kala, K. S., Mattila-Sandholm, T., Pol, I., Smid, E. J., et al. (1998). Characterization of the action of selected essential oil components on gram-negative bacteria. *Journal of Agricultural and Food Chemistry, 46*(9), 3590–3595.

Knudsen, D. M., Yamamoto, S. A., & Harris, L. J. (2001). Survival of *Salmonella spp.* and *Escherichia coli O157:H7* on fresh and frozen strawberries. *Journal of Food Protection, 64*, 1483–1488.

Lambert, R. J. W., Skandamis, P. N., Coote, P. J., & Nychas, G. J. E. (2001). A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. *Journal of Applied Microbiology, 91*(3), 453–462.

Martinez-Romero, D., Serrano, M., Bailén, G., Guillén, F., Zapata, P. J., Valverde, J. M., et al. (2008). The use of a natural fungicide as an alternative to preharvest synthetic fungicide treatments to control lettuce deterioration during postharvest storage. *Postharvest Biology and Technology, 47*(1), 54–60.

Newman, Y. K., Archbold, M. C., & Hamilton-Kemp, T. R. (2001). Survival of *Escherichia coli O157:H7* on strawberry fruit and reduction of the pathogen population by chemical agents. *Journal of Food Protection, 64*, 1334–1340.

Norman, C. (1988). EPA sets new policy on pesticide cancer risks. *Science, 242*(4877), 366–367.

Nychas, G. E., & Tassou, C. C. (2000). Traditional preservatives – oils and spices. In R. K. Robinson, C. A. Batt, & P. D. Patel (Eds.), *Encylopedia of food microbiology* (pp. 1717–1722). London, UK: Academic Press.

Palou, L., Usall, J., Muñoz, J. A., Smilanick, J. L., & Viñas, I. (2002). Hot water, sodium carbonate, and sodium bicarbonate for the control of postharvest green and blue molds of clementine mandarins. *Postharvest Biology and Technology, 24*(1), 93–96.

Sakagami, Y. M. H., Nakanishi, T., Inatomi, Y., Watabe, K., Iinuma, M., Tanaka, T., et al. (2001). Inhibitory effect of plant extracts on production of verotoxin by enterohemorrhagic *Escherichia coli O157: H7. Journal of Health Science, 47*, 473–477.

Serrano, M., Martinez-Romero, D., Castillo, S., Guillén, F., & Valero, D. (2005). The use of natural antifungal compounds improves the beneficial effect of MAP in sweet cherry storage. *Innovative Food Science & Emerging Technologies, 6*(1), 115–123.

Tzortzakis, N. G. (2009). Impact of cinnamon oil-enrichment on microbial spoilage of fresh produce. *Innovative Food Science & Emerging Technologies, 10*(1), 97–102.

Wendakoon, C., & Sakaguchi, M. (1993). Combined effect of sodium chloride and clove on growth and biogenic amine formation of *Enterobacter aerogenes* in mackerel muscle extract. *Journal of Food Protection, 56*, 410–413.

Woods, J. L. (1990). Moisture loss from fruits and vegetables. *Postharvest News and Information, 1*, 195–199.

Xu, J., Zhou, F., Ji, B. P., Pei, R. S., & Xu, N. (2008). The antibacterial mechanism of carvacrol and thymol against Escherichia coli. *Letters in Applied Microbiology, 47*(3), 174–179.

Yu, K., Newman, M. C., Archbold, D. D., & Hamilton-Kemp, T. R. (2001). Survival of *Escherichia coli* O157:H7 on strawberry fruit and reduction of the pathogen population by chemical Agents. *Journal of Food Protection, 64*, 1334–1340.