Inhibition of *Listeria monocytogenes* growth on fresh-cut produce with Thyme essential oil and essential oil compound Verbenone

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Keywords: Antilisterial treatment; Iceberg lettuce; Cantaloupe melon; pineapple; chlorine dipping

- The work is an original research carried out by the authors.
- All authors agree with the contents of the manuscript and its submission to the journal.
- All Authors listed have contributed significantly to the work and agree to be in the author list.
- The manuscript has not been previously submitted for publication or was published on a website. Aspects of the manuscript have been presented (poster and abstract in proceedings) on the Irish Branch SGM meeting in Galway (17-19th June, 2015).
- No related manuscripts to this submission have been or are currently in revision at any other scientific journal.
Highlights

- Thyme EO and Verbenone are effective anti-listerial agents on melon and pineapple
- Direct application of anti-listerial agents on fruits is more effective than dipping
- Verbenone is maintaining a high product appearance for melon while Thyme EO is not
Abstract

The anti-listerial effectiveness of Thyme essential oil (EO) and EO compounds Camphor and Verbenone was examined on fresh-cut lettuce, cantaloupe melon and pineapple with modified atmospheres and air in model packages at 4 and 8 °C. *Listeria monocytogenes* was found to be able to survive and grow in all atmospheres on melon and lettuce. However, on pineapple lowest survival was identified, presumably due to product pH. Thyme EO demonstrated the best anti-listerial effect, although direct application of the EO compromised product appearance. While Camphor showed no anti-listerial effects, Verbenone was found to have anti-listerial properties and maintained high sensory acceptance in fresh-cut fruit. The high growth rates of *L. monocytogenes* on melon were significantly reduced with the application of Verbenone while being completely eliminated on pineapple. The use of Thyme EO and Verbenone as an antimicrobial dip was successfully applied to reduce growth of *Listeria* on fresh-cut melon and eliminate growth on pineapple; however growth-reduction was less pronounced in melon when compared to a conventional chlorine dip. Further research will be necessary to optimise conditions in fresh-cut produce treatments with natural products including Verbenone and Thyme EO to replace current chlorine treatments for improved food safety.
**1 Introduction**

Ready-to-eat (RTE) fresh cut fruits and vegetables that may be consumed without further cooking or reheating can be assigned to potentially high risk foods. There is potential for contamination from seed, soil, irrigation water, organic fertilisers, harvesting, processing and packaging (Beuchat, 1996; Fonseca & Rushing, 2006; Olaimat & Holley, 2012; Ragaert, Devlieghere, & Debevere, 2007; Rico, Martín-Diana, Barat, & Barry-Ryan, 2007) and fresh-cut produce can harbour large and diverse populations of autochthonous microorganisms. Minimally processed fresh produce go through a cutting or slicing step that potentially helps pathogens to survive and grow due to access to new surfaces. Cut produce are more prone to chemical and microbiological deterioration since the cutting process is destroying cells that in turn release minerals, sugars, vitamins, and other compounds (Froder et al., 2007). These nutrients released at the damaged surfaces provide nourishment for microbes and potentially foster microbial growth.

*Listeria monocytogenes* is a Gram–positive, facultative anaerobic, rod-shaped foodborne pathogen that causes a variety of diseases in humans including septicaemia, meningitis and spontaneous abortions. In several aspects, *L. monocytogenes* is different to most known foodborne pathogens in that it is ubiquitous in the environment, resistant to various environmental conditions, such as high acid, salt and elevated levels of CO₂ and is psychotrophic (Bourke & O’Beirne, 2004; Cotter, Gahan, & Hill, 2001; Cotter, O’Reilly, & Hill, 2001; McClure, 1989). *Listeria monocytogenes* outbreaks have been linked to the consumption of a number of fresh fruit including cantaloupes and tomatoes (Colás-Medà, Abadias, Alegre, Usall, & Viñas, 2015), and due to its high case fatality rate, listeriosis ranks among the most frequent cause of death due to foodborne illness worldwide (Stephan et al., 2015).
Concerns over allergic reactions in sensitive individuals caused by traditional food preservatives such as chemical sanitizers and the potential formation of carcinogenic by-products has increased the interest in alternative, natural antimicrobial compounds (Rico et al., 2007). Aromatic plants and their extracts have the potential to become a substitute to conventional preservatives. Essential oils (EOs) and other secondary plant metabolites have been shown to possess strong antimicrobial effects against a range of foodborne pathogens such as *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus* and *Salmonella* Typhimurium (Burt, 2004; Calo, Crandall, O'Bryan, & Ricke, 2015; Scollard, Francis, & O’Beirne, 2013). EOs and their components are hydrophobic, and it is believed that this enables them to dissolve in the lipids of the bacterial cell membrane and mitochondria, rendering them more permeable and leading to the leakage of essential cell content (Burt, 2004; Lopez-Romero, González-Ríos, Borges, & Simões, 2015).

Chlorine-based sanitizers including liquid chlorine and hypochlorite are most commonly used to decontaminate fresh produce and are typically used at concentrations of 50 to 200 mg L\(^{-1}\) free chlorine at a contact time of below 5 minutes (Francis & O' Beirne, 2002). However, several studies have reported that elimination of *L. monocytogenes* from the surface of vegetables by chlorine is limited or ineffective (Nguyen-the & Carlin, 1994; Pangloli & Hung, 2013; Zagory, 1999). In addition, there are safety concerns associated with chlorine, such as its reaction with natural organic matter resulting in the formation of halogenated carcinogenic by-products (Ölmez & Kretzschmar, 2009; Zhang & Farber, 1996). Considering that fresh produce have the potential to harbour pathogenic bacteria, the development of new and effective sanitizing procedures is paramount. Studies have shown that some EOs are only effective when in direct contact with *L. monocytogenes* (López, Sánchez, Batlle, & Nerín, 2005; Scollard, Francis, & O’Beirne, 2009). However, direct contact of EOs with fresh produce can negatively impact on the sensory quality deeming the product unacceptable to
the consumer (De Azeredo et al., 2011; Scollard et al., 2013). The use of small amounts of EOs as an alternative to chlorine washes may be a way to provide the balance between sensory quality, consumer acceptability and antimicrobial efficacy. Alternatively, individual herb and EO components may have anti-listerial properties without the negative sensory impact on the fresh cut produce that includes Verbenone and Camphor that were identified to contribute to the anti-listerial effects of Rosemary (Scollard, Francis, & O'Beirne, 2014). The aim of the current study was to identify the effectiveness of Thyme EO, Verbenone and Camphor against *L. monocytogenes* on fresh-cut produce. This was carried out under different atmospheres and incubation temperatures to simulate storage abuse. Effects of the use of EO and its components as an alternative antimicrobial dip were examined to determine whether sensory appearance can be improved while still maintaining the antimicrobial effects.
2 Materials and Methods

2.1 Preparation of fresh-cut fruits (melon, pineapple) and vegetable (lettuce)

Whole cantaloupe melons (Class I Costa Rica) and pineapples (Class I Costa Rica) were obtained locally from a fruit and vegetable supplier (Richardson’s Foods, Limerick) the day before testing and stored at 4 °C prior to processing. Peels, husks, stems and cores were manually removed using a disinfected sharp stainless steel knife and cut into 25 mm pieces. Fruit pieces (100 g) were transferred into rigid polylactic acid (PLA) trays within pillow packs (20 x 20 cm) composed of a high barrier laminate film (PET12 + PE55), which were later heat-sealed. The film had a permeability to O₂ of 4.3 pmol m⁻² s⁻¹ kPa⁻¹ and to CO₂ of 17.2 pmol m⁻² s⁻¹ kPa⁻¹ (Amcor Flexibles, Gloucester, UK).

Heads of Iceberg lettuce were acquired from the same local fruit and vegetable supplier as above on the day of testing. The outer leaves alongside any damaged leaves and the core of the heads were excluded. The remaining inner leaves were sliced by hand with a disinfected sharp stainless steel knife into strips of approximately 1 cm. Portions of vegetable (25 g) were transferred into bags made from 35 μm thick oriented polypropylene packaging film (18 cm x 10 cm) which had a permeability to O₂ of 5.7 nmol m⁻² s⁻¹ kPa⁻¹ and to CO₂ of 19 nmol m⁻² s⁻¹ kPa⁻¹ (Scollard et al., 2009).

2.2 Preparation of Listeria cultures and inoculation of fruits/vegetable

For all inhibition tests a combination of three L. monocytogenes strains from the Listeria strain collection at Teagasc Food Research Centre (Moorepark, Ireland) were used in order to follow the European Guidelines for challenge tests on ready-to-eat foods (EUR Lm, 2014).

The mix comprised of a EURL Lm reference strain (number 1382), a persistent strain isolated from a food processing plant (number 6179) and a strain isolated from a vegetable production facility (number 959). The cultures were grown separately in tryptone soya broth (10 mL
TSB, Oxoid CM129, Fannin Healthcare, Cork, Ireland) at either 4 or 8 °C in accordance with European Guidelines for challenge tests, which specify that cultures should be incubated at similar temperatures to test conditions. Cultures were then mixed together to achieve equal numbers (CFU) of each strain and then diluted in phosphate buffered saline (PBS, Oxoid BR014, Fannin Healthcare) to allow inoculation of fresh-cut produce at $10^2$ CFU g$^{-1}$.

0.1 ml aliquots of Listeria suspension were distributed uniformly over the fruits/vegetable contained within each of the packages. Following inoculation, the antimicrobial treatments were applied to the fruits/vegetables and then heat sealed under defined atmospheres (see below).

### 2.3 Antimicrobial treatments

The effectiveness of the antimicrobial treatments on the survival and growth of Listeria were tested as follows:

A) **Direct antimicrobial application (spraying)**

0.5 ml of undiluted Thyme EO (60-65 % EO from supercritical fluid extraction of Thyme with carbon dioxide; Guinness Chemicals Ltd., Portlaoise, Ireland), Camphor (96 % Sigma Aldrich, 1 g ml$^{-1}$ dissolved in ethanol) or Verbenone (>93 %; Sigma Aldrich) was used as antimicrobial treatments, respectively and were sprayed over the fresh-cut products (100 g fruit or 25 g lettuce) in a fine mist using a spray bottle (Scollard et al., 2013).

B) **Antimicrobial dipping**

Antimicrobial dipping was carried out under a modified protocol (Lee & Baek, 2008) using Thyme EO, Verbenone and chlorine (free chlorine from calcium hypochlorite, as a comparison) at levels of 150 mg L$^{-1}$. Briefly, 100 g of inoculated fresh-cut fruit was placed in a metal basket and dipped for 2 min in approximately 500 mL of dipping solution at room temperature under gentle agitation. The fruit was then rinsed three times in sterile water at 4
ºC (washing step), spun to remove excess water and allowed to air dry before being packaged as detailed above (see workflow, supplementary Fig. S1).

2.4 Atmospheric treatments, package sealing and storage conditions

Following inoculation and antimicrobial treatment, packs were flushed with the following gas atmospheres: (a) air, (b) 8kPa CO₂, 4kPa O₂, 88kPa N₂ (optimum) or (c) 15kPa CO₂, 1kPa O₂, 84kPa N₂ (sub-optimum modified atmosphere packaging, MAP) and heat sealed using a vacuum packer (Multivac, UK). The packs were then stored (incubated) at either 4 or 8 ºC for up to 7 days.

2.5 Enumeration of Listeria

Bacterial cell counts were carried out on day 0 (day of inoculation) and days 2, 5, 7 (throughout storage) from four replicate packs. The fruit/vegetable samples from each package were homogenised for 120 s at high speed in phosphate buffer saline (PBS, Oxoid BR0014) in a 2 fold dilution using a Seward laboratory stomacher (Stomacher Model 400, AGB Scientific, Ireland). Following this, 2 mL of sample was centrifuged at 4000 g for 360 s (centrifugation had no effect on cell viability, data not shown). Supernatant was removed and 0.2 mL of PBS added before pellets were re-suspended and 0.1 mL aliquots were plated in duplicate on Listeria selective agar (LSA, Oxoid CM856) containing a modified Listeria selective supplement (Oxoid SR0206). The resulting detection limit was therefore 0.5 CFU g⁻¹ fruit or vegetable. As required, further serial dilutions were performed in PBS and plated on LSA during the testing period. CFU of Listeria monocytogenes (black colony with black zones around colony) were determined after incubation at 35 ºC for 48 hours.
2.7 Evaluation of sensory quality

Evaluation of appearance was performed on the fresh-cut produce packages during storage (days 0, 2, 5 and 7) by an untrained sensory panel (Scollard et al., 2013). The panel consisted of 5 evaluators (4 female and 1 male) who were members of our laboratory with experience in sensory evaluation of fresh-cut produce. Prior to analysis, panellists were familiarised with the product and scoring procedure. The panellists were asked to score the appearance of samples, on an 11 point scale ranging from 10 to 0, where 10 represented excellent appearance and 0 represented very poor appearance. The samples were coded (three random digits) and randomly offered to panellists. Panellists were given one sample and asked to evaluate colour and appearance against that of a fresh-cut sample (control). The evaluations were carried out under typical daylight conditions and at a temperature of 18 to 20 ºC.

2.8 Statistical analyses

*Listeria* counts were reported as the means of four independent values ± standard deviations. Incubation experiments with Thyme EO and EO compounds at different atmospheres and temperatures were tested for homoscedasticity (Leven’s test) and normality (Shapiro-Wilk test). Where conditions of homoscedasticity and normality were met, an ANOVA with Tukey posthoc test was conducted. Where only homoscedasticity was met, an ANOVA with Games-Howell posthoc was applied. Where both requirements were not met, even after data transformation, a Kruskal-Wallis test and manual posthoc was conducted to identify significant differences (for all tests, $P \leq 0.05$).
3 Results

3.1 Effect of direct application of Thyme EO, Verbenone and Camphor on L. monocytogenes in MAP fresh-cut lettuce (4 °C)

In lettuce, L. monocytogenes was able to survive and grow in all atmospheres tested (Fig 1a). The direct application of Thyme EO and Verbenone had a significant anti-listerial effect. No viable cells of Listeria were detected from day 0 of storage for Thyme EO (Fig 1b; detection limit: 0.5 CFU g⁻¹) and only intermittent survival of L. monocytogenes (day 2 at 1 kPa O₂ and day 5 in air, below 100 CFU g⁻¹) was found in the presence of Verbenone (Fig 1c). Camphor was found to have no significant anti-listerial effect, as growth/survival of L. monocytogenes remained similar to the control (Fig 1d). Differences among the three applied atmospheres were miniscule and mostly not significant with the exception of Camphor on day 7 (significance reached between air and 4kPa O₂ MAP) and the control on day 5 (significance reached between 1kPa O₂ and 4kPa O₂ MAP).

3.2 Effect of direct application of Thyme EO and Verbenone on L. monocytogenes in MAP fresh-cut fruit at 4 °C

On melon at 4 °C, Thyme EO significantly reduced Listeria populations (P < 0.01) with no cultivable cells being detected from day 0 to day 7 in all atmospheres (Fig 2a). The addition of Verbenone resulted in a significant reduction of viable Listeria cells over the controls at day 0, 2, 5 and 7 (non-parametric test, no separation between atmospheres). A 1 log reduction in growth over the controls on day 0 was recorded that was followed by a 1.5 log reduction by day 2. This effect increased to a 4 log reduction at suboptimal MAP (1kPa O₂) with no cultivable cells being detected by the end of storage (day 7). However, at optimum MAP (4kPa O₂) around 1 log CFU g⁻¹ of L. monocytogenes survived in the presence of Verbenone and under atmospheric condition (air) the survival rate was closer to 2 log CFU g⁻¹ melon,
thus the survival rate in Verbenone was significantly higher than in Thyme EO. Significant
differences among the applied atmospheres were only detected for days 5 and 7, where
suboptimal MAP (1kPa O₂) had the lowest counts of Listeria of all three atmospheres.

For fresh-cut pineapple at 4 °C, the addition of Verbenone and Thyme EO reduced Listeria
counts with no cultivable cells detected from day 0, and reductions of this order persisted
until the end of storage (Fig. 2b; all lines overlaid at log 0). However, control populations
were also reduced upon addition to pineapple with a 0.4 log population seen on day 0 for all
atmospheres that progressed towards detection of no cultivable cells by the end of storage,
thus differences between controls and treatments were not statistically significant at day 7
(Fig. 2b).

3.3 Effect of direct application of Thyme EO and Verbenone on L. monocytogenes in
MAP fresh-cut fruit at 8 °C

Experiments repeated for pineapple at 8 °C resulted in findings similar to the 4 °C incubation
(see section 3.2 above) with CFU g⁻¹ fruit only exceeding log 1 in the absence of Thyme EO
or Verbenone. On all days of sampling, application of Thyme EO or Verbenone significantly
reduced viable numbers of Listeria over the control. At day 7 Thyme EO, Verbenone and the
modified atmospheres reduced the number of cultivable Listeria on pineapple to below the
detection limit (Supplementary Fig. S2a).

Experiments repeated for melon at 8 °C without anti-listerial treatments resulted in growth of
L. monocytogenes in air and modified atmospheres, reaching 5 log CFU g⁻¹ melon after 7
days. The addition of Thyme EO or Verbenone significantly reduced the number of viable
Listeria for all days of sampling (Supplementary Fig. S2b). While Verbenone prevented
growth of Listeria, a bactericidal effect was not observed as on days 0 to 7 CFU g⁻¹ stayed
around 2 log. In contrast, Thyme EO significantly reduced the number of cultivable *Listeria* further (all sampling days) to below the detection limit by day 7 (Supplementary Fig. S2b).

### 3.4 Effects of EO, Camphor and Verbenone on appearance of fresh-cut produce

Direct application of Thyme EO, Camphor or Verbenone to lettuce resulted in appearance scores being reduced to ‘unacceptable’ by day 2 of storage for all treatments at 4 °C (data not shown). Consequently, further work at 8 °C was abandoned.

The addition of Thyme EO to cantaloupe melon at 4 °C (Fig. 3a) reduced appearance scores throughout storage to unacceptable levels, with levels dropping to below a score of 6 by day 2 of storage. Interestingly, at 8 °C, appearance scores with Thyme EO slipped only to a score between 7 and 8 for day 2 and 5 and only dropped to around 6 after 7 days (Supplementary Fig. S3a). The addition of Verbenone to melon at both temperatures however had no effect on the appearance scores with scores remaining similar to the control throughout storage between scores 9 and 10 (control and Verbenone lines in Fig. 3a and S3a overlaid).

Similar results were found for pineapple with Verbenone having no negative effect on appearance as scores from Verbenone and the controls of over 9 were identical throughout storage at 4 °C (Fig 3b) and 8 °C (Supplementary Fig. S3b). Furthermore, the addition of Thyme EO to pineapple only reduced the appearance by 2 points and the product remained at an acceptable level throughout storage at 4 °C. Unlike the results for melon, Thyme EO application at abuse storage temperature (8 °C) on pineapple lowered the appearance score further and dropped to around 6 by day 7.
3.5 Effects of antimicrobial dipping of fresh-cut fruit on *L. monocytogenes* in

The effects of antimicrobial dipping (chlorine, Thyme EO and Verbenone) on *L. monocytogenes* survival and growth during storage on fresh-cut fruit at 8 °C are presented in Fig. 4. In melon, chlorine, Thyme EO and Verbenone dip at day 0 significantly reduced viable counts of *L. monocytogenes* by 0.8, 0.5 and 0.2 logs, respectively. This significant effect was still present at day 2, where log reductions over the control reached 1.2, 1 and 0.8 (chlorine, Thyme EO and Verbenone, respectively). However this effect was no longer present for Thyme EO and Verbenone by day 7 of storage, where only chlorine dipping retained a significant reduction (0.9 log) over the control (Fig. 4a).

In pineapple (Fig. 4b), all three dips (chlorine, Thyme EO and Verbenone) showed significant reductions in viable *Listeria* cells of 1.5 log on day 0. This effect continued until the end of storage, where no cultivable cells were detected for chlorine, Thyme EO and Verbenone dips on days 5 and day 7. Due to reductions in viable numbers of *Listeria* in the control from day 2 onwards, the difference between the control and the treatments was no longer significant for days 2-7.
4 Discussion:

The increasing interest in the beneficial effects of essential oils has led to their inclusion in the production of numerous food products. Many EOs and their individual constituents are considered to be ‘Generally Recognized as Safe’ (GRAS) at the doses typically used in foods (Burt, 2004) and have been approved by the Food and Drug Administration (FDA) for use in edible products. Of the EO/EO compounds tested in this study Thyme EO was found to have major anti-listerial effects with no growth seen on any of the fresh-cut produce tested throughout storage with the exception of the dipping experiment. This is in agreement with previous reports which identified that Thyme EO has strong anti-listerial properties in cheese, and meat (Govaris, Botsoglou, Sergelidis, & Chatzopoulou, 2011; Solomakos, Govaris, Koidis, & Botsoglou, 2008).

Previous studies have shown that a range of chemical constituents of EOs possess antimicrobial properties in vitro (Faleiro et al., 2005; Friedman, Henika, & Mandrell, 2002). More recently, the essential oil components Camphor and Verbenone were found to have strong anti-listerial effects (Santoyo et al., 2005; Scollard et al., 2014). In this study, direct application of Verbenone was found to have a significant effect on L. monocytogenes growth with no growth seen on any of the fruit or vegetable products by the end of storage, although on some occasions a proportion of cells of L. monocytogenes remained viable. The addition of Verbenone to lettuce in air packs resulted in a final log concentration of 2 log CFU g⁻¹, hence growth was prevented but its effect was largely bacteriostatic. However, when Verbenone was added to fresh cut pineapple in air no growth was seen from day 0 of storage at 4 °C and CFUs were also greatly reduced from day 0 at 8 °C, thus a bacteriocidal effect was observed. This is presumably due to the low pH of pineapple (pH 3.4, data not shown), where the combination of Verbenone with the acidic conditions appeared to enhance the effectiveness of the EO compound (synergistic effect). Many studies have shown that low pH
on its own has a detrimental effect on the growth of *L. monocytogenes* (Cole, Jones, & Holyoak, 1990; George, Lund, & Brocklehurst, 1988) and that efficacies of essential oils are enhanced at low pH (Burt, 2004). Here, the combined effect of pH and Verbenone resulted in the maximal possible reduction of cultivable *Listeria* cells. In melon, the application of Verbenone significantly reduced the number of viable *Listeria* cells consistently to below 100 CFU g$^{-1}$, even under suboptimal atmosphere and abuse temperature. At the same time, no negative effect on product appearance was identified. This was a considerable advantage over Thyme EO that although reduced numbers of *Listeria* further, had negative effects on the appearance of melon. Consequently, the direct application of Verbenone shows promising potentials for commercial applications on melon.

Since previous *in vitro* experiments with Camphor indicated anti-listerial properties (Santoyo et al., 2005; Scollard et al., 2014), this EO component was tested in this study as well. In contrast, Camphor was found to be ineffective in reducing listerial growth in lettuce and thus was not tested in subsequent experiments. Indeed, studies by Celiktas *et al.* (2007) found that methanol extracts of Camphor exhibited very low antimicrobial activity compared to the parental EOs and were inactive against a number of microorganisms.

Studies have shown EOs to work best when in contact (Scollard et al., 2009), however this poses a threat for the sensory quality of fruits and vegetables. It was found in this study that any addition of EOs or their individual chemical components to fresh cut lettuce proved detrimental to its sensory appearance and it would not be recommended for use on this product. Phytotoxic effects of EOs on delicate plant material, such as lettuce and sliced bell peppers, have previously been reported (Scollard et al., 2013; Uyttendaele, Neyts, Vanderswalmen, Notebaert, & Debevere, 2004). While the dark Thyme EO resulted in unacceptable sensory scores in the fresh cut fruit, the addition of Verbenone had no negative
sensory effects both on melon and pineapple. This is presumably due to the clear colouring of the EO extract and the more robust structure of the fruit products.

Different atmospheres provided in the packaging in this study (air, optimal MAP and suboptimal MAP) resulted rarely in significant different abundances of surviving *L. monocytogenes* cells, with the Thyme EO and Verbenone MAP packs having very stable atmospheres. This atmosphere effect played an even lesser role at the sensory appearance testing. Indeed, studies on cantaloupe melon in MAP of different oxygen concentrations found little benefit to increase shelf life (Gomes, Beaudry, & Almeida, 2012). Since *Listeria* is a facultative anaerobe, low oxygen levels will have no impact on its survival rate.

As higher concentrations of plant EOs are generally required in foods than *in vitro*, the application of EOs in foods may be limited due to changes in the sensory quality of the food or to interactions of EOs with food components (Devlieghere *et al.* 2004). The addition of essential oils to food products improved the sensory attributes (odour and appearance) of fish and meat (Goulas and Kontominas 2007, Fernández-López *et al.* 2005), however, clove and cinnamon EOs had unpalatable effects in meats and cheeses (Lis-Balchin and Deans 1997).

Bagamboula *et al.* (2004) showed that addition of 0.1% (v/v) thyme oil (diluted in ethanol) proved detrimental to the sensory quality of lettuce leading to browning and strong odour development. Undesirable organoleptic effects might be limited by diluting EOs and using them as an antimicrobial dip. Sanitisers such as chlorine are commonly used in a number of countries to reduce the microbial load on a number of fruits and vegetables. However, some studies have shown that the chlorine concentrations (50–200 mg L$^{-1}$) that are traditionally used for decontamination are not effective in successfully reducing pathogen loads on vegetables (de Medeiros Barbosa *et al.*., 2016; Lee & Baek, 2008). In this study dipping the melon pieces in 150 mg L$^{-1}$ of chlorine led to significant reduction in surviving *Listeria* albeit only by just 0.5 log. Replacing chlorine with a more natural antimicrobial dip such as Thyme...
EO or Verbenone (150 mg L\(^{-1}\)) reduced growth significantly too for the first 2 days after dipping but significance to the control was lost after 7 days of storage. This could be due to the fact that dipping/washes are less effective on damaged or cut produce than on intact produce due to limited accessibility of bacteria to washes in damaged areas or changes in the surface of the produce that may favour bacterial adherence (Hoelzer, Pouillot, Van Doren, & Dennis, 2014). Also, the molarity of chlorine (0.004 mol L\(^{-1}\), used in this study) was four times that of the EO/EO compound (0.001 mol L\(^{-1}\)), thus a Thyme EO or Verbenone dipping treatment at a molarity equal to chlorine may be more effective. In pineapple however, again a significant treatment effect was seen with the dipping in EO and Verbenone led to a 1.5 log reduction on day 0 and no viable cells were detected by day 7 of storage. Consequently, dipping of pineapple in Thyme EO or Verbenone might provide enough synergistic effects alongside its natural low pH in order to replace chlorine dipping.

In summary, in terms of EO/EO compounds, Thyme EO displayed the strongest antimicrobial effects against *Listeria* but a negative sensory effect on most occasions, rendering it often non-applicable under the here developed experimental setups. In contrast, Verbenone had a moderate anti-listerial effect combined with a neutral sensory effect on tested fruits. At no stage direct application of Verbenone to fruit allowed *Listeria* to grow beyond EU limits (100 CFU g\(^{-1}\)), not even under suboptimal atmospheres and abuse temperature. Thus, the addition of EO compounds, such as Verbenone, may be a way to provide the balance between sensory acceptability and antimicrobial efficacy in fresh-cut fruit. There is further potential for Verbenone to be used as an alternative to chlorine as a dipping treatment, if used in combination, like in the case of pineapple where an added hurdle, such as pH, enhanced its anti-listerial properties.
Acknowledgements

We would like to thank the Irish Department of Agriculture, Food and the Marine for financing this study (FIRM 11F008).
References


