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

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Keywords: Anti-listerial 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.

Abstract

45 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
50 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
55 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
60 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 psychotropic (Bourke & O' Beirne, 2004; Cotter, Gahan, & Hill, 2001; Cotter, O' Reilly, & Hill, 2001; Mc Clure, 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, & Rieke, 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 × 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.

Aliquots of 0.1 mL 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)*

A 0.5 mL aliquot 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, 1g mL⁻¹ dissolved in ethanol) or verbenone (>93 %; Sigma Aldrich) were 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

°C (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 °C 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 x 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 °C 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 °C.

2.8 Statistical analyses

Listeria counts were reported as the means of four independent values $\pm$ 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, $P \leq 0.05$).

225 **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).

230 The direct application of thyme EO and verbenone had a significant anti-listerial effect ($P < 0.05$). No viable cells of *Listeria* were detected from day 0 of storage for lettuce treated with 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
235 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 (significant differences found between air and 4kPa O₂ MAP) and the control on day 5 (significant differences found between 1kPa O₂ and 4kPa O₂ MAP).

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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 with no cultivable cells being detected from day 0 to day 7 in all atmospheres (Fig 2a). The addition of
245 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

250 (4kPa O₂) around 1 log CFU g⁻¹ of *L. monocytogenes* survived in the presence of verbenone

and under 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.

255 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,
260 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

265 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
270 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 thyme 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 were identical (above 9) 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 an abusive 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 (typically around pH 3.4), 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 abusive 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 Celiktaş *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

365 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
370 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
375 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).
380 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. The substantial sensory quality deterioration of lettuce was also confirmed in the current study when in contact with thyme EO, verbenone and camphor.

Undesirable organoleptic effects might be limited by diluting EOs and using them as an
385 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

390 150 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 were reported to be less effective on damaged or cut produce than on intact
395 ones (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
400 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
405 non-applicable under the experimental conditions. In contrast, verbenone had a moderate anti-listerial effect combined with a neutral sensory effect on tested fruits. At no stage did direct application of verbenone to fruit allowed *Listeria* to grow beyond EU limits (100 CFU g⁻¹), not even under suboptimal atmospheres and abusive temperatures. Thus, the addition of EO compounds, such as verbenone, may be a way to provide the balance between sensory
410 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.

415 **Acknowledgements**

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

420 **5 References**

- Bagamboula, C. F., Uyttendaele, M. and 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, 33-42.
- 425 Beuchat, L. R. (1996). Pathogenic microorganisms associated with fresh produce. *Journal of Food Protection*, 59(2), 204-216.
- Bourke, P., & O' Beirne, D. (2004). Effects of packaging type, gas atmosphere and storage temperature on survival and growth of *Listeria* spp. in shredded dry coleslaw and its components. *International Journal of Food Science and Technology*, 39(5), 509-523.
- 430 Burt, S. (2004). Essential oils: Their antibacterial properties and potential applications in foods - A review. *International Journal of Food Microbiology*, 94(3), 223-253.
- Calo, J. R., Crandall, P. G., O'Bryan, C. A., & Ricke, S. C. (2015). Essential oils as antimicrobials in food systems – A review. *Food Control*, 54, 111-119.
- 435 Carlin, F., Nguyen-The, C., Da Silva, A. A., & Cochet, C. (1996). Effects of carbon dioxide on the fate of *Listeria monocytogenes*, of aerobic bacteria and on the development of spoilage in minimally processed fresh endive. *International Journal of Food Microbiology*, 32(1-2), 159-172.
- 440 Celiktaş, O. Y., Kocabas, E. E. H., Bedir, E., Sukan, F. V., Ozek, T., & Baser, K. H. C. (2007). Antimicrobial activities of methanol extracts and essential oils of *Rosmarinus officinalis*, depending on location and seasonal variations. *Food Chemistry*, 100(2), 553-559.
- Colás-Medà, P., Abadías, M., Alegre, I., Usall, J., & Viñas, I. (2015). Effect of ripeness stage during processing on *Listeria monocytogenes* growth on fresh-cut 'Conference' pears. *Food Microbiology*, 49, 116-122.
- 445 Cole, M. B., Jones, M. V., & Holyoak, C. (1990). The effect of pH, salt concentration and temperature on the survival and growth of *Listeria monocytogenes*. *Journal of Applied Bacteriology*, 69(1), 63-72.
- Cotter, P. D., Gahan, C. G. M., & Hill, C. (2001). A glutamate decarboxylase system protects *Listeria monocytogenes* in gastric fluid. *Molecular Microbiology*, 40(2), 465-475.
- 450 Cotter, P. D., O' Reilly, K., & Hill, C. (2001). Role of the glutamate decarboxylase acid resistance system in the survival of *Listeria monocytogenes* LO28 in low pH foods. *Journal of Food Protection*, 64(9), 1362-1368.
- 455 De Azeredo, G. A., Stamford, T. L. M., Nunes, P. C., Gomes Neto, N. J., de Oliveira, M. E. G., & de Souza, E. L. (2011). Combined application of essential oils from *Origanum vulgare* L. and *Rosmarinus officinalis* L. to inhibit bacteria and autochthonous microflora associated with minimally processed vegetables. *Food Research International*, 44(5), 1541-1548.
- 460 de Medeiros Barbosa, I., da Costa Medeiros, J. A., de Oliveira, K. Á. R., Gomes-Neto, N. J., Tavares, J. F., Magnani, M., & de Souza, E. L. (2016). Efficacy of the combined application of oregano and rosemary essential oils for the control of *Escherichia coli*, *Listeria monocytogenes* and *Salmonella* Enteritidis in leafy vegetables. *Food Control*, 59, 468-477.
- 465 Devlieghere, F., Vermeulen, A. and Debevere, J. (2004). Chitosan: antimicrobial activity, interactions with food components and applicability as a coating on fruit and vegetables. *Food Microbiology*, 21, 703-714.
- EURL *Lm* TECHNICAL GUIDANCE DOCUMENT for conducting shelf-life studies on *Listeria monocytogenes* in ready-to-eat foods, Version 3, complement of Regulation (EC) No. 2073/2005 (2014).

- Faleiro, L., Miguel, G., Gomes, S., Costa, L., Venâncio, F., Teixeira, A., . . . Pedro, L. G. (2005). Antibacterial and antioxidant activities of essential oils isolated from *Thymbra capitata* L. (Cav.) and *Origanum vulgare* L. *Journal of Agricultural and Food Chemistry*, 53(21), 8162-8168.
- 470 Fernández-López, J., Zhi, N., Aleson-Carbonell, L., Pérez-Alvarez, J. A. and Kuri, V. (2005). Antioxidant and antibacterial activities of natural extracts: application in beef meatballs. *Meat Science*, 69, 371-380.
- 475 Fonseca, J. M., & Rushing, J. W. (2006). Effect of ultraviolet-C light on quality and microbial population of fresh-cut watermelon. *Postharvest Biology and Technology*, 40(3), 256-261.
- Francis, G. A., & O' Beirne, D. (2002). Effects of vegetable type and antimicrobial dipping on survival and growth of *Listeria innocua* and *E. coli*. *International Journal of Food Science and Technology*, 37(6), 711-718.
- 480 Friedman, M., Henika, P. R., & Mandrell, R. E. (2002). Bactericidal activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella enterica*. *Journal of Food Protection*, 65(10), 1545-1560.
- 485 Froder, H., Martins, C. G., de Souza, K. L. O., Landgraf, M., Franco, B. D. G. M., & Destro, M. T. (2007). Minimally processed vegetable salads: Microbial quality evaluation. *Journal of Food Protection*, 70, 1277-1280.
- George, S. M., Lund, B. M., & Brocklehurst, T. F. (1988). The effect of pH and temperature on initiation of growth of *Listeria monocytogenes*. *Letters in Applied Microbiology*, 6(6), 153-156.
- 490 Gomes, M. H., Beaudry, R. M., & Almeida, D. P. F. (2012). Influence of oxygen and temperature on the respiration rate of fresh-cut cantaloupe and implications for modified atmosphere packaging. *HortScience*, 47(8), 1113-1116.
- Goulas, A. E. and Kontominas, M. G. (2007). Combined effect of light salting, modified atmosphere packaging and oregano essential oil on the shelf-life of sea bream (*Sparus aurata*): Biochemical and sensory attributes. *Food Chemistry*, 100, 287-296.
- 495 Govaris, A., Botsoglou, E., Sergelidis, D., & Chatzopoulou, P. S. (2011). Antibacterial activity of oregano and thyme essential oils against *Listeria monocytogenes* and *Escherichia coli* O157:H7 in feta cheese packaged under modified atmosphere. *LWT - Food Science and Technology*, 44(4), 1240-1244.
- 500 Hoelzer, K., Pouillot, R., Van Doren, J. M., & Dennis, S. (2014). Reduction of *Listeria monocytogenes* contamination on produce – A quantitative analysis of common liquid fresh produce wash compounds. *Food Control*, 46, 430-440.
- Lee, S.-Y., & Baek, S.-Y. (2008). Effect of chemical sanitizer combined with modified atmosphere packaging on inhibiting *Escherichia coli* O157:H7 in commercial spinach. *Food Microbiology*, 25(4), 582-587.
- 505 Lis-Balchin, M. and Deans, S. G. (1997). Bioactivity of selected plant essential oils against *Listeria monocytogenes*. *Journal of Applied Microbiology*, 82, 759-762.
- Lopez-Romero, J. C., González-Ríos, H., Borges, A., & Simões, M. (2015). Antibacterial effects and mode of action of selected essential oils components against *Escherichia coli* and *Staphylococcus aureus*. *Evidence-Based Complementary and Alternative Medicine*, 2015, 9.
- 510 López, P., Sánchez, C., Batlle, R., & Nerín, C. (2005). Solid- and vapor-phase antimicrobial activities of six essential oils: Susceptibility of selected foodborne bacterial and fungal strains. *Journal of Agricultural and Food Chemistry*, 53(17), 6939-6946.
- 515

- Mc Clure, P. J., T. A. Roberts, and P. Otto Oguru. (1989). Comparison of effects of sodium chloride, pH and temperature on the growth of *Listeria monocytogenes* on gradient plates and in liquid medium. *Letters in Applied Microbiology*, 9, 95–99.
- Nguyen-the, C., & Carlin, F. (1994). The microbiology of minimally processed fresh fruits and vegetables. *Critical Reviews in Food Science and Nutrition*, 34(4), 371–401.
- Olaimat, A. N., & Holley, R. A. (2012). Factors influencing the microbial safety of fresh produce: A review. *Food Microbiology*, 32(1), 1–19.
- Ölmez, H., & Kretzschmar, U. (2009). Potential alternative disinfection methods for organic fresh-cut industry for minimizing water consumption and environmental impact. *Food Science and Technology*, 42(3), 686–693.
- Pangloli, P., & Hung, Y.-C. (2013). Effects of water hardness and pH on efficacy of chlorine-based sanitizers for inactivating *Escherichia coli* O157:H7 and *Listeria monocytogenes*. *Food Control*, 32(2), 626–631.
- Ragaert, P., Devlieghere, F., & Debevere, J. (2007). Role of microbiological and physiological spoilage mechanisms during storage of minimally processed vegetables. *Postharvest Biology and Technology*, 44(3), 185–194.
- Rico, D., Martín-Diana, A. B., Barat, J. M., & Barry-Ryan, C. (2007). Extending and measuring the quality of fresh-cut fruit and vegetables: a review. *Trends in Food Science & Technology*, 18(7), 373–386.
- Santoyo, S., Cavero, S., Jaime, L., Ibañez, E., Señoráns, F. J., & Reglero, G. (2005). Chemical composition and antimicrobial activity of *Rosmarinus officinalis* L. essential oil obtained via supercritical fluid extraction. *Journal of Food Protection*, 68(4), 790–795.
- Scollard, J., Francis, G. A., & O'Beirne, D. (2009). Effects of essential oil treatment, gas atmosphere, and storage temperature on *Listeria monocytogenes* in a model vegetable system. *Journal of Food Protection*, 72(6), 1209–1215.
- Scollard, J., Francis, G. A., & O'Beirne, D. (2014). Chemical basis of anti-listerial effects of rosemary herb during stomaching with fresh-cut vegetables. *LWT - Food Science and Technology*, 57(1), 16–21.
- Scollard, J., Francis, G. A., & O'Beirne, D. (2013). Some conventional and latent anti-listerial effects of essential oils, herbs, carrot and cabbage in fresh-cut vegetable systems. *Postharvest Biology and Technology*, 77, 87–93.
- Solomakos, N., Govaris, A., Koidis, P., & Botsoglou, N. (2008). The antimicrobial effect of thyme essential oil, nisin, and their combination against *Listeria monocytogenes* in minced beef during refrigerated storage. *Food Microbiology*, 25(1), 120–127.
- Stephan, R., Althaus, D., Kiefer, S., Lehner, A., Hatz, C., Schmutz, C., . . . Mäusezahl-Feuz, M. (2015). Foodborne transmission of *Listeria monocytogenes* via ready-to-eat salad: A nationwide outbreak in Switzerland, 2013–2014. *Food Control*, 57, 14–17.
- Uyttendaele, M., Neyts, K., Vanderswalmen, H., Notebaert, E., & Debevere, J. (2004). Control of *Aeromonas* on minimally processed vegetables by decontamination with lactic acid, chlorinated water, or thyme essential oil solution. *International Journal of Food Microbiology*, 90(3), 263–271.
- Zagory, D. (1999). Effects of post-processing handling and packaging on microbial populations. *Postharvest Biology and Technology*, 15(3), 313–321.
- Zhang, S., & Farber, J. M. (1996). The effects of various disinfectants against *Listeria monocytogenes* on fresh-cut vegetables. *Food Microbiology*, 13(4), 311–321.

Figure legends:

Figure 1. Effects of a) gas atmosphere, b) thyme EO, c) verbenone and d) camphor on survival and growth of *Listeria monocytogenes* in MAP fresh-cut lettuce at 4 °C. Reported populations are means of 4 values. Error bars show standard deviation. Different letters identify significant differences within sampling days.

Figure 2. Effects of thyme EO and verbenone on survival and growth of *Listeria monocytogenes* in MAP fresh-cut a) melon and b) pineapple stored at 4 °C. Reported populations are means of 4 values. Error bars show standard deviation. Different letters identify significant differences within sampling days; * = verbenone data were compared as combined atmospheres via nonparametric test (Kruskal-Wallis).

Figure 3: Effects of thyme EO and verbenone on appearance scores of fresh-cut MAP (a) melon and (b) pineapple during storage at 4 °C. Reported scores are means of 4 values. Error bars show standard deviation. Different letters identify significant differences within sampling days.

Figure 4. Effects of dipping with chlorine, thyme EO and verbenone (150 mg L⁻¹) on *Listeria* survival and growth on fresh-cut a) melon and b) pineapple during storage at 8 °C. Reported populations are means of 4 values. Error bars show standard deviation. Different letters identify significant differences within sampling days.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

a)

b)