

# Study of Antimicrobial Activity of five Plant Essential Oils Against *Listeria Monocytogenes* in Sausages

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**\*Corresponding Author:** Hamdy A. Shaaban, Department of Chemistry of Flavour and Aroma, Food Industries and Nutrition Research Institute, National Research Centre, Dokki, Giza, Egypt.**Received date:** August 11, 2025; **Accepted date:** August 27, 2025; **Published date:** September 04, 2025**Citation:** Hamdy A. Shaaban, (2025), Study of Antimicrobial Activity of five Plant Essential Oils Against *Listeria Monocytogenes* in Sausages, *Clinical Research and Clinical Trials*, 13(3); DOI:10.31579/2693-4779/283**Copyright:** © 2025, Hamdy A. Shaaban. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.**Abstract:**

The antimicrobial activity of five plant essential oils were evaluated against *Listeria monocytogenes*, both in vitro and in a food system. Essential oils of thyme, clove, and pimenta were found to be most effective, based on disc diffusion experiments. Thyme and clove proved to be highly effective against *L. monocytogenes* in peptone water (1 g/l) and reduced the bacterial population below detection limits at concentrations of 1 ml/l. Experiments were also carried out in sausage of different fat content (zero-, low-, and full-fat) to evaluate the antimicrobial activity of essential oils against *L. monocytogenes*. Thyme essential oil reduced bacterial populations significantly ( $P \leq 0.05$ ) at 1 ml/l level in zero- and low-fat sausage, but not in full-fat sausages. At 10 ml/l level it reduced the bacterial population  $> 1.3 \log_{10}$  cfu/g in zero-fat sausages, but was less effective in low and full-fat sausages. Clove essential oil also exhibited antimicrobial activity at 1 ml/l in all sausages, and was more effective than thyme at 5 ml/l. However, increasing concentration to 10 ml/l did not result in significant ( $P \leq 0.05$ ) reduction of bacterial population. It is concluded that efficacy of essential oils was reduced in a food system due to interaction with food components.

**Keywords:** essential oils; *L. monocytogenes*; sausages**Introduction**

The genus *Listeria* includes 22 species (Kaszoni-Rückerl *et al.*, 2020) of which *L. monocytogenes* and *L. innocua* are the most prevalent. *L. monocytogenes* is a Gram-positive, facultative anaerobe, rod-shaped bacteria, about 0.5  $\mu$ m in width and 1–1.5  $\mu$ m in length (Gandhi & Chikindas, 2007), with the ability to multiply in a wide temperature range (2–45  $^{\circ}$ C), a wide pH range (4.6–9.5), and water activity values of at least 0.92 (Buchanan *et al.*, 2017).

*Listeria monocytogenes* causes listeriosis, a food-borne disease that occurs predominantly in pregnant women, the elderly and immunosuppressed individuals and can lead to miscarriages and death (Kim & Kathariou, 2009; Swaminathan & Gerner-Smidt, 2007). *L. monocytogenes* can be found in raw and processed foods such as milk, dairy products, meat products, seafood and vegetables (Franklin *et al.*, 2004; Nguyen *et al.*, 2008) causing many times the recall of foods with substantial economic losses to the food industry worldwide (Gandhi & Chikindas, 2007).

The Codex Alimentarius defines Ready-to-Eat (RTE) products “any food which is normally eaten in its raw state or any food handled, processed, mixed, cooked, or otherwise prepared into a form which is normally eaten without further listericidal steps” (CAC, 2007), thus taking into consideration the hazard represented by *Listeria monocytogenes*. This microorganism is a widespread environmental contaminant, due to its extreme adaptability: it can grow at 1.0 $^{\circ}$ C and pH 4.8 (Farber & Peterkin, 1991). These characteristics make many RTE products potential ideal

substrate for *L. monocytogenes* growth and a major source of listeriosis cases (EFSA, 2009 and 2010).

Minimally processed and fresh sauces possess product characteristics and other intrinsic factors that make them potential ideal substrates for the development of various microorganisms, among which *L. monocytogenes*. Contamination can occur during preparation either as a result of cross contamination due to poor hygienic conditions or of non compliance to correct processing procedures (e.g. use of time-temperature parameters) (Bertollo *et al.*, 2007; Maria Ausilia *et al.*, 2013). Very few and local studies have evaluated the presence of *Listeria* spp., in fresh sauces, such as basil pesto sauce (Magistrini, 2006), cheese and mushroom sauces (Grassi *et al.*, 2011).

The increase in bacterial resistance to antibiotics, the lack of new drug development, and increased concern about the use of synthetic additives continues to be a pressing challenge that drives the search for promising alternatives against *L. monocytogenes* (Chouhan *et al.*, 2017). Therefore, the use of natural preservatives, such as essential oils (EOs), has increased. Essential oils are complex mixtures of compounds that are synthesized by different plant parts (roots, stems, leaves, flowers, fruits, and seeds). The antibacterial properties of EOs are mainly assigned to individual components (e.g., carvacrol, thymol, p-cymene, and cinnamaldehyde) (Ali *et al.*, 2022).

Cross contamination from plant workers is also a possible cause of *Listeria* spp. getting into the food product after heat treatment (Genigeorgis *et*

al.,1990). It has been shown that *L. monocytogenes* is able to survive and grow to significant numbers on refrigerated meat products (Grau & Vanderlinde, 1992) making post-process contamination a significant concern for RTE meat products. The meat industry at present uses chemical preservatives like sodium acetate, sodium lactate and various nitrites to prevent the growth of *L. monocytogenes* in the products. Concerns over the safety of some chemical preservatives have prompted an increased interest in more natural alternatives. There is a need to find safe and effective replacements for chemical preservatives and treatments. Particular interest has been focused on the potential application of plant essential oils. Essential oils have been known to exhibit antibacterial properties for a long time now (Shelef, 1983; Deans & Ritchie, 1987; Aureli *et al.*, 1992; Lis-Balchin & Deans, 1997; Smith- Palmer *et al.*, 1998). Most studies have been conducted using essential oils in vitro using microbiological media (Ting & Diebel, 1992; Remmal *et al.*, 1993; Pandit & Shelef, 1994; Firouzi *et al.*, 1998; Hammer *et al.*, 1999; Campo *et al.*, 2000; Griffin *et al.*, 2000; Elgayyar *et al.*, 2001; Delaquis *et al.*, 2002); consequently, little is understood about their effectiveness when applied to food.

The effect of EOs on bacteria is reflected in the inhibition of their growth or the destruction of the bacterial cell (Swamy *et al.*, 2016 ) through different specific mechanisms of action. The interaction of EOs with the bacteria cells causes the degradation of the cell wall, damage to the cytoplasmic membrane, and damage to membrane proteins. This damage induces the leakage of the cell contents, the coagulation of the cytoplasm, and the depletion of the proton motive force, all causing cell death (da Silva *et al.*, 2021). A study identifying the various plant essential oils against *L. monocytogenes* and estimating its effectiveness in vitro and in a food matrix system would be of interest in exploring the possible use of these essential oils as a means to prevent food born outbreaks of listeriosis. The objective of this study was to identify the essential oils exhibiting antimicrobial properties against *L. monocytogenes*; also, to study the efficacy of essential oils as natural preservatives against *L. monocytogenes* on commercial sausages of different fat contents.

## Materials and Methods

### Microorganisms and cultures:

The three different strains of *L. monocytogenes* selected for the study, Scott A (4b), V7 (1/2a) and F5069 (4b) were obtained from Microbiological Dept. National Research Center (NRC) Dokki, Giza, Egypt. Scott A is the clinical isolate from the 1983 milk born listeriosis outbreak in Massachusetts, and V7&F5069 had been isolated from raw milk (Ryser, 1999). Individual stock cultures were maintained on tryptic soy agar (TSA) (Difco) at 4°C. Cultures were prepared by inoculating a loop of each strain into 10 ml of brain–heart infusion (BHI) broth (Difco) for 24 h at 37°C for 3 consecutive days prior to the experiments. Test cultures were placed on plate count agar (PCA) and enumerated before experiments. Cell concentration was approximately  $1 \times 10^9$  cfu/ml.

### Essential oils:

Five different essential oils that have been known to exhibit antimicrobial properties against *L. monocytogenes* were selected for the study. The essential oils of thyme (*Thymus vulgaris*), clove (*Eugenia caryophyllata*), pimenta (*Pimenta dioica*), rosemary (*Rosemarinus officinalis*), and sage (*Salvia officinalis*) were extracted by Chemistry of Flavour & Aroma Dpet.(NRC). All of the essential oils were stored in brown bottles at 4°C. Serial dilutions were prepared by adding equal quantities of ethanol (200 proof) to the essential oils just prior to the experiment to minimize any interaction between the antimicrobial components of the essential oils and ethanol.

### Disc diffusion method:

The agar diffusion method was used to detect the antimicrobial activity of the essential oils (thyme, clove, pimenta, rosemary, and sage). Culture plates were prepared with 10 ml of PCA (Difco), and were overlaid with 5ml of tryptic soy agar (soft agar) (TSA with 8.0 g agar) at  $40 \pm 1^\circ\text{C}$  containing 1ml

of bacterial culture ( $1 \times 10^9$  cfu/ml). This was done to ensure a uniform growth of the culture throughout the media plates and to provide an aerobic environment to the *L. monocytogenes*. Six sterile paper discs (diameter 6.0 mm) were placed at different locations on the surface of culture plates. Serial dilutions of different essential oils were prepared and kept at the room temperature (21°C) prior to the experiments. Five microliters of serial dilutions of essential oil suspension were dropped on each paper disc, and a disc soaked with absolute ethanol (200 proof) were used as the positive control to detect any possible antimicrobial effect due to ethanol content of the dilutions. The treated plates were incubated at  $37 \pm 1^\circ\text{C}$  for 48 h and were visually inspected for any zone of inhibition around the paper discs.

### Inhibition activity in peptone water:

Based on the disc diffusion studies essential oils of thyme, clove, and pimenta were selected for further studies in peptone water (1.0 g/l). Since, no single strain could be identified as the most susceptible to the action of the essential oils; a cocktail bacterial culture was prepared by mixing three different strains, Scott A, F5069, and V7 in the same proportion, and used for all subsequent experiments. Test tubes containing 8.9 ml of sterile peptone water (1.0 g/l) at room temperature (21°C) were inoculated with 0.1 ml of mixed strains of *L. monocytogenes* and were placed on modified Oxford agar (Difco) to enumerate cell counts. Bacterial count in peptone water was determined to be approximately  $10^7$  cfu/ml. A stock essential oil suspension with oil concentration of ten times the desired strength was prepared by adding the pre-determined essential oil quantity to sterile deionized water, and shaking vigorously by hand to ensure proper dispersion of the suspension. The concentrations of essential oils used are 0.1, 0.25, 0.5, and 1.0 ml/l. One milliliter of the stock oil suspension was added to 9 ml of the peptone water (1.0 g/l) containing bacterial culture. The test tube was shaken vigorously for the specified treatment times (5, 10, and 15 min) on a standard test tube shaker (Deluxe Mixer, IL, USA) intermittently to ensure proper dispersion of the essential oils in the suspension. Sterilized water (1 ml) was added to the test media to act as the control for this experiment. Serial dilutions were spread plated (0.1 ml) on plates. After incubation of plates for 48 h at 37°C, presumptive *L. monocytogenes* colonies were counted and confirmed as being *Listeria* positive using API *Listeria* Kit (BioMerieux Inc., USA).

### Beef sausages as model food for antimicrobial study:

Effect of essential oils as antimicrobial agents was studied on the commercially available sausages. Beef sausages with three different levels of fat contents (zero-fat, 0.0 g/kg; low-fat, 90.0 g/kg; and full-fat, 260.0 g/kg) were procured from local stores and were kept frozen ( $-18^\circ\text{C}$ ) in the Department of Food Technology, (NRC) for the duration of the experiments. A representative sample of sausages of different fat content was taken and analyzed for pH using pH meter. The pH of the sausages ranged from 6.3 (zero-fat) to 6.9 pH units (full-fat). Ten milliliters of bacterial culture of each strain (Scott A, F 5069, and V7) ( $10^9$  cfu/ml) were mixed and diluted with 270 ml of sterile deionized water. The cocktail bacterial culture was placed on PCA and enumerated before each set of experiments. The bacterial suspension had cell strength of  $10^8$  cfu/ml. Two sausages (45–55 g each) were dipped in the bacterial suspension (300 ml) for 2 min, and were continuously stirred by hand to make sure that proper attachment of bacteria took place on the sausages surface. The excess bacterial suspension was drained off and the sausages were air-dried in the class II biosafety cabinet for 3 h at 21°C to dry out any excess moisture present on the sausages surface. Dried samples were then kept overnight in a refrigerator ( $4 \pm 1^\circ\text{C}$ ) to ensure sufficient bacterial attachment. The inoculated sausages were sliced in half (23–27 g) using sterile knife and were used for the experimental purposes. The essential oil suspensions of desired strength (1.0, 5.0, and 10.0 ml/l) were prepared by adding measured essential oil quantities in sterilized water and shaken vigorously. Inoculated sausages samples (23–27 g) were placed in a stomacher bag, and essential oil suspension equal to four times in volume of its weight was added into the bag. The stomacher bag was then kept in a plastic container that was continuously stirred at 150 rpm in a shaker at room temperature (21°C) for the specified treatment time (5, 10, or 15

min). After shaking, the essential oil suspension was drained off the stomacher bag. Treated samples were rinsed with sterile water (100 ml) to wash off the adhered oil suspension. Samples were then gently mass aged for 2 min in a stomacher bag containing 100 ml sterile peptone water (1.0 g/l) to release the attached cells into the liquid exudates. Inoculated untreated samples acted as positive control. Samples were also treated with sterilized water for the same treatment period to act as wash control. Samples were analyzed for populations of *L. monocytogenes* as described previously.

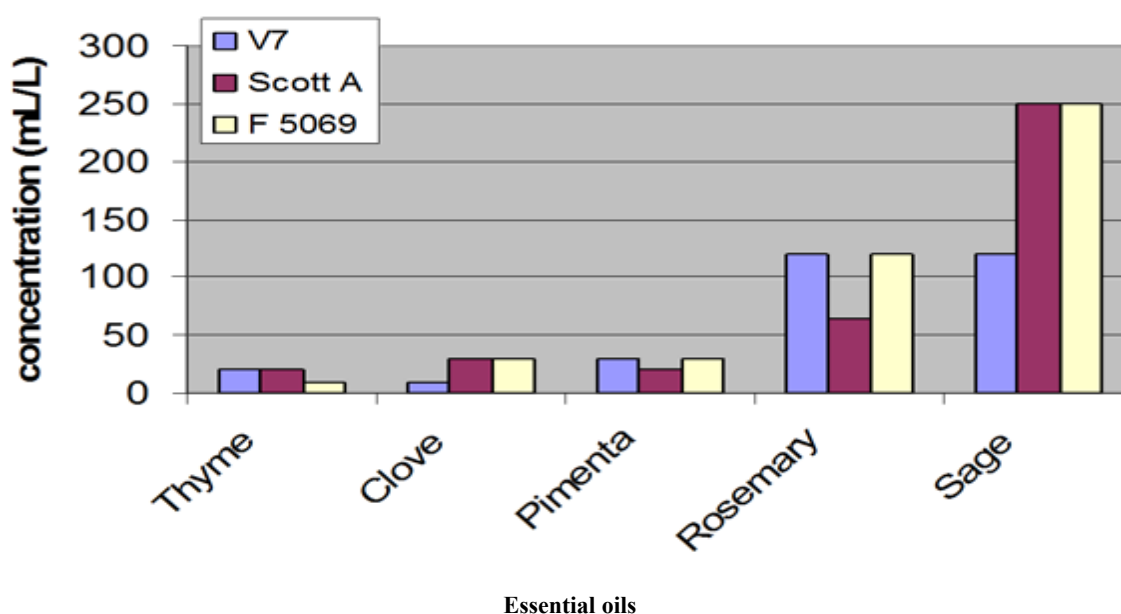
### Statistical analysis:

All experiments were replicated three times. The differences in mean were calculated using the Duncan's multiple-range tests for means with 95% confidence limit ( $P \leq 0.05$ ). Pair-wise difference of means was calculated using Tukey's *t*-test. Statistical analysis of the data was done using the SAS software. Each value presented represents a mean of six values (duplicate values from each sample from three replicate trials).

### Results and Discussion

Preliminary experiments were conducted in vitro using the disc diffusion method to investigate the antimicrobial action of the essential oils of thyme,

clove, pimenta, rosemary, and sage (Fig. 1). Thyme oil was the most inhibitory essential oil against all strains of *L. monocytogenes*. It produced a clear zone of inhibition against strain F 5069 at concentration of 7.8 ml/l, and 15.6 ml/l for Scott A and V7. Clove and pimenta were also inhibitory against *L. monocytogenes* resulting in a clear zone of inhibition at levels of 31.2 and 15.6 ml/l on various strains. However, rosemary and sage were not very effective as they produced a clear zone of inhibition at relatively high levels of 62.5–125.0 and 125.0–250.0 ml/l, respectively. These results indicate that no particular strain could be determined to be most susceptible or most resistant to the antimicrobial action of the various essential oils. Kim, *et al.*, (1995) noted that in addition to the zone of inhibition being dose dependent, bacterial strains respond differently to the action of various essential oils. Although the exact mechanism of action of essential oils is not known, it is believed to be due to change in the cell membrane structure causing leakage of various enzymes and nutrients (Cox *et al.*, 2000). On the basis of these results, thyme, clove, and pimenta essential oils were selected for determining the minimum inhibitory concentration (MIC) against mixed cocktail culture of three strains of *L. monocytogenes* in peptone water (1.0 g/l) as rosemary and sage required relatively high levels of concentration to inhibit *L. monocytogenes* in disc diffusion tests.



**Figure 1:** Minimum inhibitory concentrations (MICs) of different essential oils against three strains of *L. monocytogenes*.

#### Effect of thyme, clove, and pimenta in peptone water:

**Table (1)** shows the effect of thyme, clove, and pimenta essential oils (concentration and treatment time) on the population of *L. monocytogenes* in peptone water (1.0 g/l). It is evident that at level 0.1 ml/l, there is no significant ( $P \leq 0.05$ ) effect of the thyme oil upon the survival of *L. monocytogenes* in peptone water from that of control samples. Also, at this level, there is no effect of treatment time with the population recovered after 5, 10, and 15 min of treatment. Most drastic effect was observed at the concentration level of 0.5 ml/l, where bacterial population was reduced to 1.98 log<sub>10</sub> cfu/g after 5 min. Further, increasing the treatment time to 10 and 15 min caused the bacterial population to decrease below the detection limit. At 1.0 ml/l concentration level, no viable bacteria could be detected for any treatment time combination. Thus, it can be concluded that the antimicrobial effect of thyme essential oil is dependent on the concentration as well as the treatment time. In another study, the minimum bacteriocidal concentration

of thyme against *L. monocytogenes* was found to be 0.3 ml/l (Smith-Palmer *et al.*, 1998). However, in that study the experiments were done in tryptic soy broth (TSB), and the tubes after the addition of essential oils were kept for 24 h. The increased treatment time of 24 h compared to 5–15 min in this study might be a factor resulting in the variation of MIC levels. Aureli *et al.* (1992) studied the effect of thyme essential oil against *L. monocytogenes* in saline solution (9.0 g/l). They reported that bacterial growth dropped below detection limits after 4 h, when 1 ml/l of thyme essential oil was added to the different bacterial strains. Duncan's test of means showed that 10 min treatment time was significantly ( $P \leq 0.05$ ) different from 5 min, but not with 15 min. Therefore, only 5 and 10 min, treatment times were chosen for clove and pimenta essential oils. Clove and pimenta oil (0.25 ml/l) did not exhibit any inhibitory action against *L. monocytogenes* irrespective of the treatment time. However, with clove oil (0.5 ml/l) the bacterial population was reduced to 1.29 and 0.97 log<sub>10</sub> cfu/g after 5 and 10 min, respectively.

| Essential oil | Treatment time (min) | Control    | Population recovered (log <sub>10</sub> cfu/g) <sup>a</sup> Concentration (ml/l) |            |            |            |
|---------------|----------------------|------------|--|------------|------------|------------|
|               |                      |            | 0.1  | 0.25       | 0.5        | 1.0        |
| Thyme         | 5                    | 8.16±0.05a | 8.16±0.04a   | 8.13±0.04a | 2.78±0.30c | 0±0.0d     |
|               | 10                   | 8.16±0.05a | 8.14±0.03a   | 8.05±0.06a | 0±0.0d     | 0±0.0d     |
|               | 15                   | 8.16±0.05a | 8.13±0.06a   | 7.86±0.08b | 0±0.0d     | 0±0.0d     |
| Clove         | 5                    | 8.17±0.03a | ND   | 8.12±0.04a | 2.19±0.19b | 0±0.0c     |
|               | 10                   | 8.16±0.03a | ND   | 8.16±0.04a | 1.87±0.75b | 0±0.0c     |
| Pimenta       | 5                    | 8.04±0.04a | ND   | 8.06±0.07a | 7.76±0.06b | 4.87±0.45c |
|               | 10                   | 8.04±0.04a | ND   | 8.07±0.02a | 7.82±0.01b | 4.45±0.15d |

Population means in each column or row within the same essential oil treatment with no letters in common are significantly different ( $P \leq 0.05$ ).

ND: not determined.

a Values are mean±SD population recovered (log<sub>10</sub> cfu/g) (n=3).

**Table 1:** Effect of thyme, clove, and pimenta essential oil (concentration and treatment time) on the survival of *L. monocytogenes* in peptone water

Whereas, a slight reduction ( $<1.0$  log<sub>10</sub> cfu/g) in viable cell population was observed with pimenta oil. It should be noted that a significant effect of treatment time was observed with thyme oil (0.5 ml/l), which is absent in the case of clove or pimenta essential oil. Thus, it can be concluded that in case of clove or pimenta, the essential oil concentration plays a more dominant role and treatment time has little effect on inhibiting the population of *L. monocytogenes*. In another study at similar concentrations, clove oil was able to suppress the growth of *L. monocytogenes* faster than thyme oil (Aureli et al., 1992). No such comparison could be made from the available data in this case. Duncan's test of means showed that with clove or pimenta oil (0.25 ml/l), the bacterial population recovered was not significantly different from the control samples. However, further increase in concentration to 0.5 and 1.0 ml/l, resulted in a significant decrease in population compared to control sample and between different concentration levels. The MIC for clove oil is 1.0 ml/l and also the same with thyme, but has lower bacteriocidal activity at 0.5 ml/l concentration level. These results are in agreement with the results of Smith-Palmer et al. (1998), who noted that the MIC of clove was greater than that of thyme against *L. monocytogenes*. MIC of pimenta essential oil in peptone water (1.0 g/l) against *L. monocytogenes* is  $>1.0$  ml/l. Pimenta essential oil has been reported to reduce *L. monocytogenes* populations in saline water below detection limits within 4 h at 1.0 ml/l, with a very drastic reduction within the first hour (Aureli et al., 1992). Smith-Palmer et al. (1998) have also reported the MIC of pimenta essential oil against *L. monocytogenes* to be the same as that of clove essential oil. However, in this study the efficacy of pimenta essential oil in reducing the bacterial

population was not that significant. Thyme and clove were able to reduce the bacterial population significantly at levels of 0.5 ml/l and were bacteriocidal at 1.0 ml/l. It has been observed earlier also that the antimicrobial activity of essential oils might vary considerably since a number of factors like the botanical source of the plant, time of harvesting, stage of development, method of extraction can significantly affect the active constituents of the essential oils (Janssen et al., 1986). Also the variation in the strains being tested and the bacterial load has some effect on the results (Remmal et al., 1993). Based on these results, it is concluded that thyme and clove essential oil have better antimicrobial activity against *L. monocytogenes* in peptone water. Both of these essential oils were completely inhibitory to the growth of *L. monocytogenes* at levels 1.0 ml/l. However, pimenta essential oil is not that effective in reducing the bacterial load in peptone water. Therefore, thyme and clove essential oils were selected to be further tested in a food matrix system of sausages for their efficacy in reducing the population of *L. monocytogenes*.

#### Effect of thyme on sausages:

Populations of *L. monocytogenes* on sausages (zero-, low-, and full-fat) surviving after treatments with thyme essential oil are shown in Table 2. Thyme oil exhibits antimicrobial activity even at 1 ml/l concentration in zero-fat sausages, the population is significantly ( $P \leq 0.05$ ) different from control at this level for 5 and 10 min treatment time. No significant difference was observed between 10 and 15 min. Bacterial population is further reduced with increase in oil concentration to 5 ml/l, resulting in a reduction of 0.67–1.05 log<sub>10</sub> cfu/g.

| sausages <sup>a</sup> | Treatment time (min) | Control     | Population recovered (log <sub>10</sub> cfu/g) <sup>b</sup> Thyme oil concentration (ml/l) |              |              |
|-----------------------|----------------------|-------------|--|--------------|--------------|
|                       |                      |             | 1.00   | 5.00         | 10.0         |
| Zero-fat              | 5                    | 6.17±0.00a  | 5.70±0.11b   | 5.12±0.01ed  | 4.84±0.22ed  |
|                       | 10                   | 5.84±0.13b  | 5.12±0.36ed  | 4.83±0.14ef  | 4.53±0.27f   |
|                       | 15                   | 5.52±0.23bc | 5.30±0.16cd  | 4.85±0.18ef  | 4.66±0.03f   |
| Low-fat               | 5                    | 6.50±0.01a  | 5.81±0.32bcd   | 5.87±0.03bcd | 5.86±0.02bcd |
|                       | 10                   | 6.13±0.02b  | 5.84±0.06bcd   | 5.72±0.10cde | 5.47±0.40e   |
|                       | 15                   | 6.14±0.09b  | 5.95±0.23bc  | 5.86±0.05bcd | 5.53±0.21ed  |
| Full-fat              | 5                    | 6.64±0.22a  | 6.49±0.24ab  | 6.20±0.05bcd | 6.09±0.07cde |
|                       | 10                   | 6.44±0.16a  | 6.47±0.06ab  | 6.08±0.20cde | 6.02±0.16cde |
|                       | 15                   | 6.44±0.10ab | 6.31±0.16bc  | 5.91±0.19ed  | 5.85±0.09b   |

a Fat content in sausages: zero-fat (0 g/kg), low-fat (90 g/kg), full-fat (260 g/kg). Initial inoculated population of *L. monocytogenes* on sausages: 5.71 log<sub>10</sub> cfu/g. Control: Population of *L. monocytogenes* after washing with sterilized water.

b Values are mean±SD population recovered (log<sub>10</sub> cfu/g) (n=3). Population means in each column or row within the same sausages sample with no letters in common are significantly different ( $P \leq 0.05$ ).

**Table 2:** Effect of thyme essential oil (concentration and treatment time) on the survival of *L. monocytogenes* in sausages (zero-, low- and full-fat)



At the maximum oil concentration tested (10 ml/l), the population is reduced by 0.86–1.33 log<sub>10</sub> cfu/g. It should be noted that at 1 ml/l concentration level, the bacterial population was reduced below the detection limit in the peptone water experiments. Effect of essential oils is known to be reduced in a food matrix system as a result of interaction with the different components of food, thus requiring much larger concentrations to reduce the bacterial populations (Farbood *et al.*, 1976; Smith-Palmer, *et al.*, 2001). In low-fat sausages, only a slight decrease in recovered population was observed when the thyme oil concentration was increased to 5 ml/l (0.28–0.63 log<sub>10</sub> cfu/g reduction), and 10 ml/l (0.61–0.66 log<sub>10</sub> cfu/g reduction). Therefore, the increase in fat content from zero- to low-fat, caused an appreciable reduction in the antimicrobial activity of the thyme essential oil as observed in the population of *L. monocytogenes*. The effect of thyme oil concentration and treatment time, on the recovered population of *L. monocytogenes* in full-fat sausages shows that there is no significant ( $P \leq 0.05$ ) difference between populations at 1 ml/l concentration and the control samples. The reduction observed is in the range of 0–0.15 log<sub>10</sub> cfu/g. Upon increasing the concentration to 5 ml/l, a slight decrease in population was observed (0.44–0.53 log<sub>10</sub> cfu/g reduction), which was not significantly ( $P \leq 0.05$ ) different from population recovered when the oil concentration was increased to 10 ml/l (0.42–0.59 log<sub>10</sub> cfu/g reduction). It should be noted that in case of zero fat sausages log reductions >1.30 were observed at similar concentrations tested. Therefore, it is evident from the results that the antimicrobial activity of thyme essential oil is greatly reduced in the presence of fat present in sausages. Similar observations were also reported earlier by Smith-Palmer *et al.* (2001). They found that the composition of cheese was shown to be an important factor in determining the effectiveness of the plant essential oils.

#### Effect of clove oil on sausages:

| sausages <sup>a</sup> | Treatment time (min) | Control    | Population recovered (log <sub>10</sub> cfu/g) <sup>b</sup> clove oil concentration (ml/l) |             |             |
|-----------------------|----------------------|------------|--|-------------|-------------|
|                       |                      |            | 1  | 5           | 10          |
| Zero-fat              | 5                    | 6.60±0.21a | 5.61±0.07bc  | 5.45±0.14bc | 5.57±0.37bc |
|                       | 10                   | 6.77±0.07a | 5.89±0.33b   | 5.06±0.46c  | 5.05±0.75c  |
| Low-fat               | 5                    | 6.70±0.00a | 6.04±0.08b   | 5.68±0.16bc | 5.57±0.16bc |
|                       | 10                   | 6.85±0.05a | 5.87±0.23b   | 5.30±0.65c  | 5.66±0.10bc |
| Full-fat              | 5                    | 7.13±0.12a | 6.74±0.08b   | 5.92±0.05d  | 5.85±0.04d  |
|                       | 10                   | 6.98±0.23a | 6.40±0.25c   | 5.92±0.04d  | 5.82±0.06d  |

**Table 3:** Effect of clove essential oil (concentration and treatment time) on survival of *L. monocytogenes* population in sausages (zero-, low- and full-fat)

Population means in each column or row within the same sausages sample with no letters in common are significantly different ( $P \leq 0.05$ ).

<sup>a</sup> Fat content sausages: zero-fat (0 g/kg), low-fat (90 g/kg), full-fat (260 g/kg). Initial inoculated population of *L. monocytogenes* on sausages: 6.12 log<sub>10</sub> cfu/g. Control: Population of *L. monocytogenes* after washing with sterilized water.

<sup>b</sup> Values are mean±SD population recovered (log<sub>10</sub> cfu/g) (n=3).

This result is different from those observed in zero- and low-fat sausages. It is possible that longer contact time between *L. monocytogenes* and clove essential oil for 10 min helped to overcome the protection offered by excess fat present in the sausages to the cell wall of *L. monocytogenes*. Whereas, in case of zero- and low-fat sausages, the maximum antimicrobial action might have already occurred before 10 min of exposure, thereby resulting in no significant difference beyond that time. Although bacterial population was reduced as a result of essential oils, the antimicrobial action was highly dependent on the fat content and somewhat on the treatment time. At concentration levels of 10 ml/l, the resulting reduction in population was <1.5 log<sub>10</sub> cfu/g and even less at 5 ml/l concentration. Clove essential oil was much more effective than thyme at all the levels tested. It had a lower MIC level than thyme in sausages of different fat contents. The treatment time had no significant effect on the reduction of *L. monocytogenes* populations in sausages. Therefore, essential oils alone cannot provide complete protection against pathogens in sausages.

#### Conclusions:

Based on the preliminary disc diffusion tests, the minimum inhibitory concentrations (MICs) of various plant essential oils ranged from 7.8 to 250

ml/l against different strains of *L. monocytogenes* tested. Thyme, clove, and pimenta proved to be more effective in inhibiting the bacterial growth in vitro than rosemary and sage. In peptone water, the essential oils had ability to significantly ( $P \leq 0.05$ ) reduce the bacterial population of *L. monocytogenes* even at relatively low levels of 0.5 ml/l. A reduction of 5–6 log<sub>10</sub> cfu/g was observed at this level in case of thyme and clove. Pimenta however, was not as much effective in reducing the bacterial load. At 1.0 ml/l levels the bacteria were reduced below detection limits by thyme and clove, and a 3 log<sub>10</sub> cfu/g reduction was observed for pimenta. In the experiments done with sausages, although bacterial population was reduced as a result of essential oils, the antimicrobial action was highly dependent on the fat content. At concentration levels of 10 ml/l, the resulting reduction in population was <1.5 log<sub>10</sub> cfu/g and even less at 5 ml/l. Clove essential oil was much more effective than thyme at all the levels tested. The treatment time had no significant effect on the reduction of populations in sausages. Therefore, essential oils alone cannot provide complete protection against pathogens in sausages. The antimicrobial action of essential oil has been known to depend on the bacterial load and might prove to be more effective in food products that have a lower level of bacterial contamination. Alternatively, they can be used as a means to increase the bacterial ‘‘hurdle’’

in products that have a strong flavour, thereby reducing the resultant organoleptic properties. Most promising application would be the use of plant essential oils in conjunction with other preservation techniques like combination with chemical preservatives, low-temperature, low-O<sub>2</sub>, high-pressure techniques to develop a synergistic alternative to current methods.

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