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# Antimicrobial activity of clove (*Syzgium aromaticum*) oil in inhibiting *Listeria monocytogenes* on chicken frankfurters

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**Abstract**

The ability of *Listeria monocytogenes* to survive and grow at refrigeration temperature in some ready to eat (RTE) poultry products is a public health concern. The inhibitory effect of clove oil (1% and 2%, v/w) applied to the surface of RTE chicken frankfurters was determined on seven strains of *L. monocytogenes* inoculated at low ($10^2$–$10^3$ cfu/g) or high cell numbers ($10^4$–$10^6$ cfu/g), and stored at 5 °C for 2 weeks or at 15 °C for 1 week. All strains of *L. monocytogenes* survived and grew on control frankfurters at 5 °C and 15 °C but growth was inhibited under both storage conditions in the presence of either 1% or 2% clove oil. Depending on the sensory considerations, the addition of clove oil to frankfurters may be an effective strategy to control *L. monocytogenes* in chicken frankfurters.


*Keywords:* Ready-to-eat; Refrigeration; Herb; *Listeria monocytogenes*

## 1. Introduction

*Listeria monocytogenes* (*L. monocytogenes*) has been isolated from a variety of foods including dairy, poultry and meat products (Broseta, Diot, Bastian, Riviere, & Cerf, 2003) and has been implicated in a number of foodborne outbreaks (Czuprynski, Faith, & Steinberg, 2003; Kathariou, 2000). An important factor contributing to the organism's foodborne disease potential is its growth at refrigeration temperature (Cressy, Jerrett, Osborne, & Bremer, 2003). Ready-to-eat (RTE) meat products have been introduced for the convenience of consumers; however, many have few barriers to microbial growth. Frequently, refrigeration is the only barrier for these types of foods, and temperature abuse at any of the links of the supply chain from the processing plants to the consumer's refrigerator, could accelerate the growth of *L. monocytogenes*. Most RTE foods receive little or no final heat treatment before being consumed because such foods are assumed to be, and often labeled as, fully cooked (Hao, Brackett, & Doyle, 1998). There have been reported illnesses resulting when supposedly RTE foods were not reheated before consumption (Pinner et al., 1992; Schuchat, Swaminathan, & Broome, 1991). There have also been a large number of recalls of RTE meat due to contamination by *L. monocytogenes* (Kathariou, 2000).

One of the greatest challenges confronting the food industry is control of *L. monocytogenes* contamination and propagation in RTE meats. After the heat processing, strict quality control is maintained till packaging (and subsequent refrigeration) to eliminate any contamination. Nonetheless, because *L. monocytogenes* can thrive in the environment of meat processing facilities, RTE meats can be contaminated with *Listeria*. In many RTE meats, *L. monocytogenes* can survive well at

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refrigeration temperature and grow at higher rates during temperature abuses, which may be encountered during warehouse storage, transportation, retail display, consumer transportation and consumer storage at home. *Listeria* survival and growth may be controlled by the addition of a herb extract or a combination of herb extracts as ingredients in RTE meat.

Herbs are frequently used as food ingredients. Herbs are bactericidal and have broad-spectrum activity against Gram-positive and Gram-negative bacteria (Baydar, Ozkan, & Sagdic, 2004; Dorman & Deans, 2000; Friedman, Henika, & Mandrell, 2002). Clove oil, a herbal extract, contains eugenol which when tested on various agar media has antimicrobial properties and has been shown to inhibit *L. monocytogenes*, *Campylobacter jejuni*, *Salmonella Enteritidis*, *Escherichia coli* and *Staphylococcus aureus* (Beuchat, 2000; Cressy et al., 2003; Smith-Palmer, Steward, & Fyfe, 1998). These tests were performed on various agar media, while on food samples the effect of the essential oils may be different. Only a few studies have been conducted to determine the antimicrobial activity of herbs on *L. monocytogenes* in actual food products (Hao et al., 1998; Yuste & Fung, 2002). The researchers have generally investigated only one or two strains of *L. monocytogenes*. No study investigating the antimicrobial effect of clove oil on multiple strains of *L. monocytogenes* on RTE chicken frankfurters could be found in the literature surveyed.

The objective of this study was to determine: (i) survival and growth of different food and clinical isolates of *L. monocytogenes* on chicken frankfurters stored at 5 °C and 15 °C and (ii) the efficacy of clove oil in inhibiting *L. monocytogenes* growth on chicken frankfurters stored at 5 °C and 15 °C.

## 2. Methods

### 2.1. Sample preparation

Chicken frankfurters were purchased at a local supermarket and brought immediately to the laboratory. The fat content of the frankfurters (97% fat free) was 3% and the pH was 6.8. The frankfurters were cut into 1-g portions and the surface of each sample was exposed to UV light ($\lambda = 260$ nm) for 10 min to kill surface contaminants.

### 2.2. Preparation of L. monocytogenes

Seven strains of *L. monocytogenes* were used (Table 1). Bacterial cultures were maintained frozen in broth at −20 °C until use. Prior to use, the cultures were activated by three successive transfers in tryptic soy broth (Difco Laboratories, Detroit, Mich.) at 30 °C for 24 h. Cells were harvested by centrifugation (10,000*g* for 10 min at 4 °C), washed three times and resuspended in phosphate buffered saline (PBS, pH 7.2). Cell numbers were determined by surface plating on tryptic soy agar in duplicate. Colonies were enumerated after 24 h of incubation at 37 °C. A final inoculum was prepared by serially diluting in PBS depending upon the desired cell numbers.

Table 1
Isolation source and serotype of *Listeria monocytogenes* strains

| Strain | Isolation source | Serotype |
|---|---|---|
| H9666 | Blood, human clinical[a] | 1/2c |
| Scott A | Human clinical, milk outbreak[a] | 4b |
| H7550 | Human clinical, hot dog outbreak[a] | 4b |
| G3982 | Human clinical, Mexican style cheese outbreak[a] | 4b |
| 12443 | Clinical isolate from monkey[b] | 1/2a |
| H7776 | Frankfurter isolate[c] | 4b |
| 101M | Beef and pork sausage isolate[c] | 4b |

[a] Strains provided by Centers for Disease Control and Prevention's *Listeria* Reference laboratory.
[b] Strains isolated from monkey at Yerkes National Primate Research Center and reported by Smith et al. (2003).
[c] Strains provided by US Department of Agriculture.

### 2.3. Treatments

Chicken frankfurters (1 g each) were inoculated with 0.1 ml of either low (ca. $10^3$) or high (ca. $10^6$) populations of different strains of *L. monocytogenes* so that the final cell numbers on frankfurters were ca. $10^2$ or ca. $10^5$ cfu/g, respectively. To the treated samples either 0.01 ml or 0.02 ml (1% or 2% v/w, respectively) of clove oil (*Syzgium aromaticum*; Citrus and Allied Essences Ltd., Lake Success, New York), was deposited on the surface using a pipette and then spread with a sterile bent glass rod. Controls consisted of chicken frankfurters inoculated with *Listeria* strains with no clove oil. Inoculated samples in petri-dishes were left undisturbed for 30 min to allow residual moisture to be absorbed, and were then stored at 5° or 15 °C and enumerated for *L. monocytogenes* at 0, 4, 8 and 14 (at 5 °C) or 0, 1, 3 and 7 (at 15 °C) days of storage. Three replications of the treatments were performed.

### 2.4. Microbial analysis

On each sampling day, two samples from each inoculation level of every *L. monocytogenes* strain and clove oil concentration were assayed. Corresponding control samples were also assayed. PBS (9 ml) was added to each sampling bag and the contents were macerated in a Stomacher (Laboratory blender, Stomacher 400) for 2 min. Samples were serially diluted (1:10) and 0.1 ml was spread plated in duplicate onto modified Oxford agar (MOX) and tryptic soy agar (TSA). MOX and TSA plates were incubated at 35 °C for 24 h to determine the population of *Listeria*. Selected presumptive