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BRIEF ARTICLE

Survival of the bacterium *Listeria monocytogenes* (Listeriaceae) after addition of lemon juice or sodium hypochlorite

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The bacterium *Listeria monocytogenes is* widely distributed in nature, and survives for up to twelve years in association with plants (Farber & Peterkin 1991). It has been detected on lettuce, cabbage, potatoes, radishes and cucumbers (Rocourt 1989) as well as on pre-cut, packaged salad vegetables (Petran *et al.* 1988, Beuchat & Brackett 1991). It causes opportunistic infections, especially in immunosuppresed patients. Several *Listeria* outbreaks have been associated with consumption of vegetables, and infection has 20-30% lethality (Rocourt 1989).

Few reports about its presence in food were available before 1986, but was later found also in foods such as dairy products and meats. In Costa Rica, a 20% prevalence was found in fresh salads (Monge y Arias 1995).

This work determines the survival of *L. monocytogenes* in fresh chopped cabbage treated with lemon juice or chlorine in several concentrations and times, simulating the preparation conditions and consumption times of Costa Rican homes.

Preparation is based on a standardized questionnaire filled by 130 Costa Rican housewives. The most common methods were the addition of juice from one lemon (aproximatelly 20 ml) or chlorinated water to the salad

For the lemon treatment, three replications, four cabbage salads were prepared according to the quantities used for a 4 - 5 members family (500 g). Each was inoculated with 10^6 CFU/g of *L. monocytogenes* (ATCC 7644) followed by 20 ml of fresh lemon juice. The salad was homogenized with a Stomacher for 15s. Immediately after homogenization and again at 5, 10, 15 and 20 min, 25 g samples of the mixture were taken, neutralized with NaOH lM to pH 7.0, placed in 225 ml of alkaline peptone water (APW), and homogenized for another 15s. Ten-fold dilutions were prepared, plated in Oxford agar and incubated for 48 h at 35°C (Vanderzant & Splittstoesser 1992). Four salads similarly prepared but without lemon were used as controls.

Four additional salads (three replications) inoculated with *L. monocytogenes* were treated with commercial sodium hypochlorite diluted to final concentrations of 5, 10, 15 and 20 mg/l, according to a titration done with mercury

nitrate, Bromathology Laboratory, Instituto Costarricense de Investigación en Nutrición y Salud (INCIENSA). Each salad was immersed in the sodium hypochlorite solution and after 5, 10, 15 and 20 min, a 25 g sample was taken and free chlorine was neutralized with sodium thiosulphate as described in the Standard Methods of Water and Wastewater (1985). Each sample was placed in 225 ml of alkaline peptone water (APW) and homogenized for another 15 s. Ten-fold dilutions were prepared, plated in Oxford agar and incubated for 48 h at 35°C (Vanderzant & Splittstoesser 1992). Four salads inoculated with *L. monocytogenes* not disinfected with chlorine and treated the same way were used as controls.

The average pH of salads without added lemon juice was 4.5. and 3.3 after lemon juice addition. Five minutes after treatment with lemon juice, *L. monocytogenes* numbers decreased from 10^6 CFU/g to 10^5 CFU/g. Later there was no change in the number of survivors, even though the sample was kept in contact with lemon juice for longer periods (up to 20 min). The levels of *L. monocytogenes* in the untreated salads did not change.

In the sodium hypochlorite treated salads, regardless of the concentrations used, *L. monocytogenes* numbers decreased from 10^6 CFU/g to 10^5 CFU/g (average) after 5 min of treatment and to 10^3 CFU/g after 20 min of treatment (Fig 1).

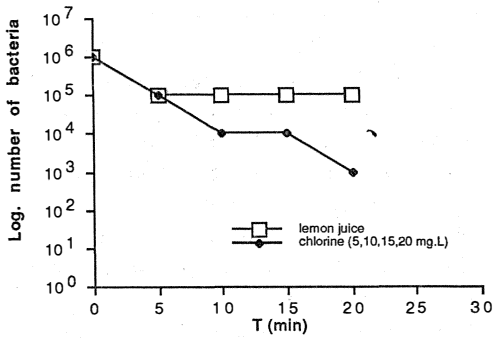

Fig. 1. Survival of *L. monocytogenes* to added lemon juice or chlorine at diferent times.

Some studies have shown that *L. monocytogenes* can grow in slightly acidic foodstuffs, but would be unable to tolerate the environment of highly acidic foods for extended periods of time (Corner *et al.* 1986). In this study, however, the average pH of the salads was highly acid (3.3), and yet the bacteria survived. Beuchat and Brackett (1991) obtained similar tolerance to acid pH when they evaluated the survival of *L. monocytogenes* in tomato juice and chopped tomatoes. Survival of *L monocytogenes* under acid conditions could be explained on the basis of Gram positive cell wall resistance to these conditions, as demonstrated by Aubrey *et al* (1994).

The efficacy of chlorine in killing *L. monocytogenes* in inoculated food has been evaluated using high disinfectant concentrations for short contact periods (Conner *et al.* 1986). Nevertheless, we decided to study the effect of lower concentrations of chlorine for longer periods of time, in order to maintain the organoleptic characteristics of vegetable salads and to simulate home practices by Costa Rican housewives.

Chlorine was more effective than lemon juice in reducing bacterium levels, but it did not achieve complete elimination. As the infective dose for *L. monocytogenes* is not known, bacterial numbers after chlorine treatment should be viewed as hazardous.

Numbers of *L. monocytogenes* found on fresh vegetables are generally less than 200 CFU/g (Farber & Peterkin 1991). At this population numbers, lemon juice and chlorine could be efficient in disinfecting them. However, as *L. monocytogenes* can grow to high numbers at refrigeration temperatures (Farber & Peterkin 1991), the disinfecting activity of lemon and chlorine would not be sufficient, as shown in the present study. This implies that the inclusion of raw vegetables or salads in the menu of immunocompromised patients should be restricted to avoid potential infections.

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