Heat resistance of *Listeria monocytogenes* in sterile distilled water in the presence of nanoemulsion of *D*-limonene

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Abstract

Nanoemulsions of oil essentials prevent microbial growth and even improving antimicrobial effect than when applied directly. Currently, there are no many studies where nanoemulsified essential oils have been combined with other factors of stress for the microorganism. This study demonstrates an interesting combined effect when applied heat and *D*-limonene nanoemulsion greatly reducing the value D significantly. These results could be of interest in the food industry to decrease thermal treatments and avoid the deterioration of the food due to the heat.

Keywords: Nanoemulsions, *D*-limonene, heat resistance, *Listeria monocytogenes,* antimicrobial effect.

1. Introduction

Listeria monocytogenes is a very ubiquitous microorganism, which is widely distributed in many environments, and can contaminate a wide variety of foods. Immunocompromised individuals, pregnant women and the elderly are those population groups most susceptible to this foodborne pathogen microorganism [1]. The ubiquity of this organism and its ability to grow in the form of biofilms enables it to be present in food processing plants and foods, being ready-to-eat foods those most likely to be contaminated [2].

One of the objectives of the present food industry is to provide consumers with better sensorial quality foods, while keeping food safety. One way to achieve this goal is to combine conventional thermal treatments with the use of natural antimicrobials. Among all of them, essential oils have gained special importance in recent years. Numerous studies have been developed evaluating the effect of the oils against a variety of microorganisms, but only a few documented studies have evaluated the combined effect of oils essentials nanoemulsions with other factors of stress for the microorganism. In 2014, Severino et al. [3] evaluated the antimicrobial effect of some essential oils with different combined non-thermal treatments (ozonized water, ultra violet-C light and gamma irradiation) against *L. monocytogenes*. Severino et al. [4] also studied the antimicrobial effect of an essential oil nanoemulsion combined with modified atmosphere packaging and gamma irradiation against *E. coli* and *Salmonella*. In both cases, interesting results were obtained. So far, there are no documented studies that have tested the antimicrobial effect of a combination of antimicrobial nanoemulsions with heat treatments.

For this reason, the aim of this study was to evaluate the combined effect of a thermal treatment with a nanoemulsion of *D*-limonene on the inactivation of *L. monocytogenes* in sterile destilled water (SDW).

2. Materials and methods

2.1 Bacterial strains

Listeria monocytogenes CECT 4032 was used in this study and it was provided by the Spanish Type Culture Collection (CECT, Valencia, Spain). This strain was stored at -80°C (30% glycerol) until use. For growth and survival experiments, fresh cultures of *L. monocytogenes* were prepared by inoculating a loop of the cryopreserved culture in tryptic soy broth (TSB; Scharlau Chemie S.A., Barcelona, Spain) and incubating overnight at 37ºC until the stationary growth phase was reached.

2.2 Antimicrobials

D-limonene was obtained from Sigma Aldrich Chemie (Steinheim, Germany). For their direct addition to sterile destilled water, they were dissolved in ethanol (Panreac, Barcelona, Spain) at 95% (v/v). The working solution was prepared

to a final concentration of 1M and stored refrigerated until use.

2.3 Preparation of nanoemulsions

The nanoemulsions of *D*-limonene were prepared following the protocol described by Maté et al. [5] and based on catastrophic phase inversion (CPI) method [6].

2.4 Heat treatments

Thermal inactivation kinetics for *L. monocytogenes* in sterile destilled water supplemented with 0.5 mM *D*-limonene (direct addition or nanoemulsion) was determined at constant temperature (52.5^{o}) in a thermoresistometer Mastia as described by Conesa et al. [7]. Surviving cells were enumerated in tryptic soy agar (TSA, Scharlau Chemie). Plates were incubated for 24 h at 37 ^{o}C. Each treatment was assayed by triplicate in independent experiments performed in different days.

2.5 Data analysis

Decimal reduction times (D-values) were calculated as the inverse negative of the slope of the regression line of the survival curves, drawn plotting the logarithm of the survivors versus the corresponding heating times. Survival curves included the average of the D values obtained for each experiment. D values are represented as mean and standard desviation (Excell 2010).

3. Results and discussion

When not antimicrobial was applied to the heating medium, *L. monocytogenes* $D_{52.5^{o}}$ value was 32.63 mins. In the case of the direct application of *D*-limonene, $D_{52.5^{o}}$ value was 22.58 mins. This date shows some additive effect by adding the antimicrobial directly to the heating medium. Finally, when the nanoemulsion of *D*-limonene was added to the heating medium, $D_{52.5^{o}}$ value was 0.31, this means it was about 100 times less than the control and 75 times less than when d-limonene was applied directly. This data shows that applying the *D*-limonene emulsified form, increases the antimicrobial effect of the same when combined with heat.

4. Conclusions

These findings reveal a striking made which could lead to important applications in the food industry. Some research had shown increased the efficiency of essential oils when they are emulsified. But not many studies have combined this technology with other factors of stress for microorganisms. The possibility of decreasing heat treatment in the food industry, achieving the same levels of food safety, could be an important advance in food processing technology.

5. Acknowledgements

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6. References

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Tables and figures

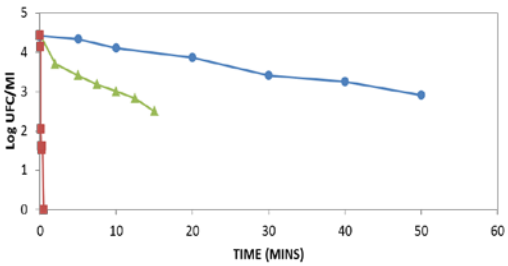

Figure 1. *Survival curves of Listeria monocytogenes in SDW at 52.5ºC. Control: ●; 0.5 mM D-limonene added directly: ▲; 0.5 mM D-limonene nanoemulsified: ■.*

Table 1. *D values (mean and standard desviation) of Listeria monocytogenes in SDW with 0.5 mM D-limonene added directly or nanoemulsified.*

Heat Medium	*Mean $D_{52.5º}$ (min) ± SD*
TSB	*32.63 ± 1.07*
TSB + 0.5 mM limonene (direct)	*22.58 ± 0.89*
TSB + 0.5 mM limonene (nanoemulsified)	*0.31 ± 0.03*