ANTILISTERIAL ACTIVITY OF CITRAL ON GROWTH KINETIC OF *LISTERIA INNOCUA*

A. B. Silva-Angulo[1], S. F. Zanini[2,*], M. Sanz-Puig, A. Rosenthal[3], A. Martínez[4], D. Rodrigo[4]

[1]Departamento de Biotecnologia Agrolimentaria, Biopolis S.L., Avda. Agustín Escardino, 9, 46980 Paterna, Spain. [2]CNPq Postdoctoral Fellowship. Espirito Santo Federal University, Department of Veterinary Medicine, Alegre, Brazil. [3]Embrapa Food Technology, Av. das Américas 23020-470, Rio de Janeiro, RJ, Brazil. [4]Department of Preservation and Food Quality, Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC), Valencia, Spain.

**Corresponding author: Tel.: +55 283 552 8653. E-mail: surama@cca.ufes.br*

Abstract. *Food industry requires the design of food preservation treatments capable of ensuring microbial inactivation to reach food safety and increase shelf life. Among emerging food preservation technologies, natural antimicrobial agent is becoming a worldwide recognized alternative to thermal processing. The aim of this work was to evaluate the antilisterial activity of citral on growth kinetic of Listeria innocua (CECT 910T). The bacterial growth rates were obtained from viable cell counts on Tryptone Soya Agar supplemented with 0.6% yeast extract (TSA-YE).The tested concentrations of citral ranged from 0.150 to 0.250 µL/mL. Control samples of bacterial growth were evaluated on the absence of citral. Experimental data were fitted to the modified Gompertz equation. The kinetic parameters of bacterial growth were characterized based on the time needed to environment adaptation (λ) and on the maximum specific growth rate (μ_{max}). Studies carried out using 10^6 cfu/mL inoculum size of Listeria innocua indicated that λ and μ_{max} were influenced by the concentration of citral ($p \leq 0.05$). As the concentration of citral increased from 0.150 µL/mL to 0.250 µL/mL an increment of λ of 2.851 hours and a decrease of μ_{max} of 0.348 log/h were observed ($p \leq 0.05$). In conclusion, it was observed that the citral showed an inhibitory effect on the kinetic growth of Listeria innocua therefore it can be a natural preservative for food products.*

Keywords. Citral, natural antimicrobials, lag phase, maximum specific growth rate, *Listeria innocua*.

Introduction

The safety and shelf life of many fresh and lightly preserved food products is dictated by the time required for pathogenic or spoilage microorganisms to reach a critical level. Thus, for predictive modelling, it is essential to determine the growth rate of those organisms. *Listeria* spp. was selected due to their relevance to a wide range of food products. It grows under refrigeration and survives in freezing environments (Gandhi &

Chikindas, 2007), whereas most other pathogenic bacteria do not grow and multiply at temperatures below 4 ºC. Among emerging food preservation technologies, natural antimicrobial agent is becoming a worldwide recognized alternative to thermal processing. Arguably the most interesting area of application for essential oils compounds is the inhibition of growth and reduction in numbers of the serious food borne pathogens such as *Listeria spp., Salmonella* spp. and *E. coli* strains. Antimicrobial activity is one of the most important properties in many compounds of essential oils. An example of such a natural antimicrobial compound is citral. While inhibition of the growth of several pathogens by citral has been reported in various articles none of these articles has addressed to evaluate the growth kinetic. The aim of this work was to evaluate the antilisterial activity of citral on growth kinetic of *Listeria innocua* (CECT 910T).

Materials and methods

Chemicals

Citral 95% was purchased as a mixture of cis and trans isomers (3,7-dimethyl-2,6-octadienal) from Sigma Aldrich Company Ltd. Dimethyl sulfoxide (DMSO) was used as dilution agent for obtaining the levels of 0.150 and 0.250 µL of citral/mL.

Bacterial strain and growth conditions

The strain of *Listeria innocua* (CECT 910T) was obtained from a pure lyophilized culture supplied by the Spanish Type Culture Collection. During this investigation, stock culture at concentration of about 7.5 x 10^8 colony forming units (cfu/mL) was maintained in cryovials at -80 °C. Bacterial broth subcultures from stock cultures were prepared by inoculating 200 µL of *Listeria innocua* in a test tube containing in 6 mL of Tryptone Soya Broth (TSB; Scharlab Chemie S.A., Barcelona, Spain) and incubated at 37 C for 12 h. The microbial suspension was mixed and diluted to obtain an inoculum size of about 1-2 x 10^6 cfu/mL at time 0.

Determination of antilisterial activity

Briefly, it were added to a sterile tube, 20 mL of TSB, 150 µL of citral at levels of 0.150 or 0.250 µL/mL and 5 mL of bacteria which were kept in a condition of incubation at 37 ºC under agitation. Control samples of bacterial growth were evaluated on the absence of citral. Culture samples of the microorganism suspension were subsequently

withdrawn every 60 minutes, up to population reached the stationary phase. The bacterial growth rates were obtained from viable cell counts data. At least, four repetitions were conducted.

Counts of viable cells and kinect parameters of bacterial growth

After treatments, the growth curves were estimated from viable plate count on Tryptone Soya Agar (TSA; Scharlab Chemie S.A., Barcelona, Spain) supplemented with 0.6% yeast extract (TSA-YE). For the curves obtainment, samples of the culture were diluted in buffered peptone water (Scharlab Chemie S.A., Barcelona, Spain) and spread in plates containing TSA-YE. The plates were incubated at 37 ºC for 48 h after which the number of colony forming units was determined. After incubation, colonies were counted by image analyzer automatic counter. To determine the kinetic of microbial growth was used non-linear regression of GraphPadPrism v. 4.03, CA, USA. The experimental data were filtered to the modified Gompertz equation (Gibson *et al.,* 1987) to determine the maximum specific growth rate (μ_{max}) and the lag phase duration (λ).

Statistical analysis

Analysis of variance (ANOVA) was conducted using Statistical Graphics System Centurion software (Statgraphics®) Centurion XV (StatPoint Technologies Inc., Virginia, USA). Fisher's LSD test was used to compare the mean values of data ($p \leq 0.05$).

Results and discussion

Studies carried out using 10^6 cfu/mL inoculum size of *Listeria innocua*, as target microorganism, indicated that λ and μ_{max} were influenced by the concentration of citral as antimicrobial agent ($p \leq 0.05$). The control cells showed maximum specific growth rate and the lag phase duration of the 0.568 log/h and 1×10^{-7} hours, respectively. As the concentration of citral increased from 0.150 µL/mL to 0.250 µL/mL an increment of λ of 2.851 hours and a decrease of μ_{max} of 0.348 log/h were observed ($p \leq 0.05$). *Listeria innocua* is a non-pathogenic microorganism but for many characteristics such as growth and biochemical characteristics it is similar to pathogenic *Listeria monocytogenes* and therefore a useful surrogate for the foodborne pathogen *L. monocytogenes*. It fills this role because in addition to its very similar physiology and metabolism with *L. monocytogenes*, the non-pathogen is equally resistant to low pH, drying, heating and salt. Such hardiness makes *L. innocua* an excellent indicator in

inoculated pack studies at food processing plants. Therefore it has been used as a model organism in various studies (Francis & O'Beirne, 1998). Our results corroborate previous finding that verified the growth inhibition of *L. monocytogenes* by citral (Kim *et al.,* 1995). Citral is an acyclic unsaturated oxygenated monoterpene aldehyde consisting of isomers, geranial and neral. The mechanism of action of monoterpenes mainly involves toxic effects to the structure and function of cell membrane (Sikkema *et al.,* 1995). It has been reported that aldehyde group conjugated to a carbon to carbon double bond present in citral is a highly electronegative arrangement, which may interfere with electron transfer during biological processes (Moleyar and Narasimham, 1986). *Listeria* spp. possesses an electron transport chain and generates energy by respiration (Seeliger & Jones, 1986).

Conclusions

In conclusion, it was observed that the citral showed an inhibitory effect on the kinetic growth of *Listeria innocua* therefore it can be a natural preservative for food products.

Acknowledgements

This work was supported by CNPq (Postdoctoral Fellowship Programme), Embrapa Labex Europa and CSIC-IATA with provided S. F. Zanini a grant to carry out this investigation.

References

Francis, G.A., O'Beirne, D. 1998. Effects of the indigenous microflora of minimally processed lettuce on the survival and growth of *Listeria innocua*. Int. J. Food Sci. Technol. 33: 477–488.

Gandhi, M, Chikindas, M.L. 2007. *Listeria*: A foodborne pathogen that knows how to survive. *International Journal of Food Microbiology* 113: 1–15.

Gibson, A.M., Bratchell, N. and Roberts, T.A. 1987. The effect of sodium chloride and temperature on the rate and extent of growth of *Clostridiurn botulinum* type A in pasteurized pork slurry. *J. Appl. Bacteriol* 62: 479-490.

Kim, J., Marshall, M.R, Wei, C.I. 1995. Antibacterial activity of some essential oil components against five foodborne pathogens. *Journal of Agricultural and Food Chemistry*, 43: 2839–2845.

Moleyar, V., Narasimham, P. 1986. Antifungal activity of some essential oil components. *Food Microbiology* 3: 331–336.

Seeliger, H., and Jones D.. 1986. Genus *Listeria*, p. 1235–1245. In: P. H. A. Sneath, N. S. Mair, M. E. Sharpe, and J. G. Holt (ed.), Bergey's manual of systematic bacteriology vol. 2. Williams and Wilkins, Baltimore, Md.

Sikkema, J, De Bont, J.A.M., Poolman, B. 1995. Mechanisms of membrane toxicity of hydrocarbons. *Microbiological Review* 59: 201–222.