

4. Conclusions and Recommendations

In this study, it was found that thyme essential oil does have an antimicrobial effect against *Listeria monocytogenes* Scott A and *Escherichia coli* ATCC 25922 at 10°C and 20°C. The minimal inhibitory concentration of thyme essential oil against these microorganisms was greater for *L. monocytogenes* than for *E. coli* and greater in fresh cheese (*in-vivo*) than in an agar system (*in-vitro*). The latter mainly because of the porous surface of the cheese, its higher amount and quality of nutrients and the more humid environment. Additionally, it was determined that the higher the temperature of storage the higher the minimal inhibitory concentration. This could be attributed either to the fact that the effectiveness of the essential oil is lower the higher the temperature, or to the fact that at a higher temperature is more feasible for microorganisms to grow and therefore more essential oil is needed to stop their growth. *Escherichia coli* ATCC 25922 in fresh cheese stored at 10°C was simply inhibited by the temperature; however, several days are needed for that, while with the spreading of cheese with thyme essential oil, at 0.22 µl thyme essential oil/g cheese, *E. coli* growth was rapidly inhibited. On the other hand, the growth of *Listeria monocytogenes* Scott A in fresh cheese stored at 10°C wasn't inhibited even applying the thyme essential oil at 0.44 µl thyme essential oil/g cheese (more than twice the *in-vitro* minimal inhibitory concentration).

Thus, it is concluded that thyme essential oil can be used successfully as a natural preservative in Mexican fresh cheese, stored at a temperature of 10°C or lower, at a concentration of at least 0.22 µl thyme essential oil/g cheese for inhibiting the growth of *E. coli*. Regarding the use of thyme essential oil as antimicrobial against *L. monocytogenes*, definitive conclusions cannot yet be made, because although it seems that thyme essential oil does have a negative effect on *L. monocytogenes* growth, additional tests are required to observe if at higher concentrations the essential oil manages to inhibit significantly its growth in fresh cheese, and determine that this concentration doesn't have any organoleptic effects.

For future studies, it is recommended to find, from the start, the minimal inhibitory concentration directly in the studied food system and not *in-vitro*, since other concentrations of essential oil should be tested (like the 2MIC in our case) that might work *in-vivo* but then one does not know if the tested concentration is really the minimal inhibitory concentration or just a concentration that inhibits the growth of the microorganisms because it is above the minimal.

Additionally, for future similar studies it is recommended to place half the volume of essential oil at the minimal inhibitory concentration on one side of the cheese and the other half on the other side. This because on the agar system the essential oil at the minimal inhibitory concentration is placed only on one side of the agar. So, in this way the results could be more comparable.

Furthermore, for future studies it is recommended to test higher concentrations of the thyme essential oil for the inhibition of *Listeria monocytogenes* Scott A in fresh cheese stored at 10°C, because even with twice the *in-vitro* minimal inhibitory concentration found in this study, the reduction of the viable *L. monocytogenes* in the cheese was not important, so at this concentration the thyme essential oil is not really useful as preservative. However, it is

important to take care of the sensory characteristics of the cheese, because an excess of essential oil could negatively affect it, and there is no point in having a cheese free of microorganisms which consumers do not like.

Finally, for the future continuation of this study it is recommended to add the thyme essential oil directly to the milk during cheese making, as Moro et al. (2014) did in their study in which they used rosemary essential oil and attained a total inhibition of clostridial species.

Sensory tests should be performed also, prior to recommend the use of essential oils in any food product. Additionally, further studies are required to evaluate the practicality of this method and its cost.