Preparation of rosemary essential oil-loaded multiple lipid nanoparticles (RO-MLNs) and evaluation of their antibacterial activity

Ben Khalifa Rayhane[1], Soraya Rodriguez Rojo[2], Frédéric Gaspar[3], Chekir-Ghedira Leila[1]
[1] Laboratory of Cellular and Molecular Biology, Faculty of Dental Medicine, University of Monastir, Avenue Avicenne, 5019 Monastir, Tunisia, benkhalifarayhane@yahoo.com; leila.chekir@laposte.net.
[2]BioEcoUVa, Institure of Bioeconomy. High Pressure Processes group, School of Industrial Engineering, University of Valladolid, Spain, soraya.rodriguez@uva.es.
[3] iBET – Instituto de Biologia Experimental e Tecnológica, Food & Health Division, Food Safety and Microbiology Laboratory, Apartado 12, 2781-901 Oeiras, Portugal, fgaspar@ibet.pt.

Introduction:
Antibacterial resistance has emerged as one of the major threatens for public health in this century mainly due mainly to antibiotic (ATB) misuse in human medicine but also in farming [1]. The combined use of ABTs with other natural antimicrobial agents, such as essential oils (EOs),provides a promising solution towards combating multidrug resistant (MDR) bacteria [2].

Owning to the failure of antibiotic therapy and the limitations of industrial applications of EOs despite their numerous biological and medicinal properties, the co-nanoencapsulation of EOs with ATBs seems to be apromising strategy to protect the active molecules against their degradation, to improve their bioavailability and their stability during the treatment and storage processes. Currently, numerous methods of encapsulation of EO have been studied and a series of more complex and structured emulsions have been developed. One of the systems that is getting increased attention in the last decade is the multiple lipid nanoparticles (MLNs) since they combine the advantages of water-in-oil-in-water (W/O/W) multiple emulsions and solid lipid nanoparticles (SLNs) to be able to co-encapsulate an EO and a hydrophilic antibiotic.

The objective of this research is to prepare a MLN suspension of pure Rosemary EO (RO-MLN) to evaluate its antimicrobial activity against MDR strain of *Pseudomonas aeruginosa*.

Methods

MLN are produced by a two step process based on melt-emulsification technique combined with ultrasonication as described elsewhere [3-5].The selected lipids are the solid glyceryl tristearate (GTS) and the liquid glyceryl trioleate (GTO). Pluronic L64 and Tween 80 were used as surfactants in the primary and secondary emulsions, respectively. The effect of different parameters in the encapsulation efficiency, particle size distribution and physical stability was studied. Regarding the primary water in oil (w/o) emulsion, the GTS was fixed at 10%, based on preliminary experiments, while varying the % EO in the liquid lipid mixture from 20% to 100%. Also the ratio of the first emulsion to the second water phase was varied from 20 to 40%. Finally, the effect of both surfactant concentrations was analyzed in the range from 0 to10% for primary emulsion and 0 to 5% for secondary emulsion

The antibacterial activity was carried out following the microdilution method using different strains of *P. aeruginosa* resistant or not to the antibiotics.

Results

The optimized RO-MLN formulation exhibited a monomodal particle size distribution with a mean particle size of 110 nm and a span <1 and encapsulation efficiency up to 90%, determined by GC-MS. RO-MLN was physically stable for at least 30 days thanks to its high negative zeta potential (> -60 mV).

The antibacterial test against susceptible and resistant strains of *P.aeruginosa* showed that RO-MLN had 6 times more antibacterial activity than the pure essential oil, which suggests that converting the essential oil to nano-scale particles improved its bacteriostatic activity.

Conclusions

The current study revealed that the RO-MLN can be considered as a new strategy to fight against bacterial multidrug resistance. The antibacterial activity could be further improved with the co-encapsulation of a drug antibiotic, such as cefepime, an aqueous soluble antibiotic.

References:

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Acknowledgements:

The authors acknowledge the financial support from Fundación General de la Universidad de Valladolid (Spain), PIP 063/147181. Ben Khalifa Rayhane thanks University of Monastir (Tunisia) for her alternation scholarship 2017-2018).