MilkQua

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Executive Summary

Background	To preserve their antimicrobial activities, EOs need to be encapsulated in suitable delivery systems compatible with food applications. Indeed, the encapsulation aims to protect essential oils from environmental conditions, reduce their toxicity, and limit their strong flavor and taste. Moreover, the encapsulation of these components also makes it possible to facilitate their homogeneous incorporation into different food matrices by reducing their hydrophobicity. With this respect, the encapsulation of EOs in nanoemulsions arises as an attempt to improve the delivery and the stability of preservatives to food products. The delivery in nanoemulsions seems to ameliorate the efficacy of EO active compounds in areas of the food matrix where target pathogenic bacteria are preferentially located. Because of their nano-sized droplet that increases the active surface area, nanoemulsions are assumed to have a superior antimicrobial activity than conventional emulsions with significantly higher droplet size.
Objectives	The main objective of this deliverable is to compare the microbial growth inhibition capacity (estimated according to an inhibition diameter on petri dishes) of bulk or free EO to its nanoemulsion. This comparaison was assessed against four bacterial and fungi strains.
Methods	***Disc Diffusion Method.*** Antimicrobial activity testing was done according to Vuddhakul et al. (2007) with slight modifications. The inocula of each microorganism were streaked, using a sterile swab, onto Mueller-Hinton agar plates for bacteria and onto Sabouraud agar plates for yeast. Then, sterile filter discs (diameter 6 mm, Whatman paper N°5) were impregnated with EO or NE and placed on the inoculated agar. After incubation (37°C for 18–24 h), the diameter of the inhibition zones around each disc was taken. The selected strains for this assay were: *Staphylococcus aureus* and *Enterococcus feacalis* (Gram positive), *Salmonella thyphimirium* and *Pseudomonas aeruginosa* (Gram negative) and the fungal strain *Candida albicans.* Each experiment was carried out 6 times and the mean diameter of the inhibition zone was recorded in mm. According to Trabelsi et *al*., 2010, inhibition zone <7 mm: Weak antimicrobial activity; inhibition zone = 7 mm: Slight antimicrobial activity, inhibition zone 8–9 mm: Moderate antimicrobial activity, inhibition zone 10–11 mm: High antimicrobial activity, inhibition zone 12–15 mm: Strong antimicrobial activity, inhibition zone > 15 mm: Excellent antimicrobial activity. ***Statistical analysis.*** For the antimicrobial tests, six replicates were used. Means were compared using the Newman-Keuls (SNK) test at a level of $p <$ 0.5 when significant differences were found by the statistical package SAS 9.1 (2002, 525).
Results and implications	It can be seen from table 1 that *T. capitatus* EO is exhibiting slight to weak antimicrobial activity againt the five organism tested, with IZ comprised between 6 and 8.67 mm (*Salmonella thyphimirium* and *Enterococcus feacalis*, respectively) and equal to 7.67 against *Candida albicans*. Interstingly, all the inhibition zones were statistically bigger when EO was encapsulated into nanoemulsion delivery system. For example, the inhibition zone of *Staphylococcus aureus* growth raised from 7 (Weak antimicrobial activity) to 10.33 mm (high antimicrobial activity). Moreover, and according

to table 1, for each bacteria/fungi, the IZ raised significantly and reached 12.33 mm in the case of *Candida albicans.*

Table 1. Growth inhibition zones (IZ, expressed as mm) obtained with *Thymus capitatus* essential oil and its nanoemulsion against five microbial strains (four bacteria and one candida). Values followed by the same letter are not significantly different at $p < 0.05$.

	IZ (mm)	
	EO	**NE**
Staphylococcus aureus	7,00± 0,46 **b**	10,33± 0,27 **d**
Enterococcus feacalis	8,67± 0, 6 **c**	10,33± 0,36 **d**
Salmonella thyphimirium	6,00± 0,27 **a**	8,67± 0,67 **c**
Pseudomonas aeruginosa	7,67± 0,58 **cd**	8,33± 0,77 **c**
Candida albicans	7,67± 0,44 **cd**	12,33± 0,46 **e**

For a better data exploitation, the percentage of the augmentation of the inhibition zone were calculated and detailed in table 2. First of all, it can be seen that the encapsulation of essential oil ameliorated its efficiency against all the tested strains. This amelioration was quite importante and varied from 8.7% (*Pseudomonas aeruginosa*) to reach 60.9% in the case of *Candida albicans.* Against *Salmonella thyphimirium* and *Staphylococcus aureus* the amelioration was estimated at 44 and 47% while against *Enterococcus feacalis* the IZ augmented by around 20%.

Table 2. Percentage of improvement of the antimicrobial efficafy (four bacteria and one candida) of *Thymus capitatus* essential oil after nanoemulsification.

	% enhancement
Staphylococcus aureus	**+ 47,62**
Enterococcus feacalis	**+ 19,23**
Salmonella thyphimirium	**+ 44,44**
Pseudomonas aeruginosa	**+ 8,70**
Candida albicans	**+ 60,87**

The data obtained from the antimicrobial essays, confirm the remarkable interst of encapsulating properly *T. capitatus* essential oil into nanoemulsion delivery system.