



β -Cyclodextrins and Liposomes as encapsulating vectors of Oregano (*Origanum vulgare* L.) essential oil: Evaluation of their antimicrobial activity against *Listeria monocytogenes* and *Salmonella* spp. in “Kefir Milk” and “Katiki Domokou” cheese

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INTRODUCTION

Oregano essential oil (OEO) is a well-recognized natural antibacterial agent with strong antimicrobial effect against foodborne pathogens such as *L. monocytogenes* and *Salmonella* spp. Its hydrophobicity, strong flavor, and high volatility impede its commercial application in food preservation. Encapsulation of OEO into matrixes such as β -cyclodextrins and liposomes protects it from oxidation and volatilization and is considered an alternative antimicrobial application. Additionally, encapsulation of OEO may enhance its bioactivity by providing a controlled and targeted release.



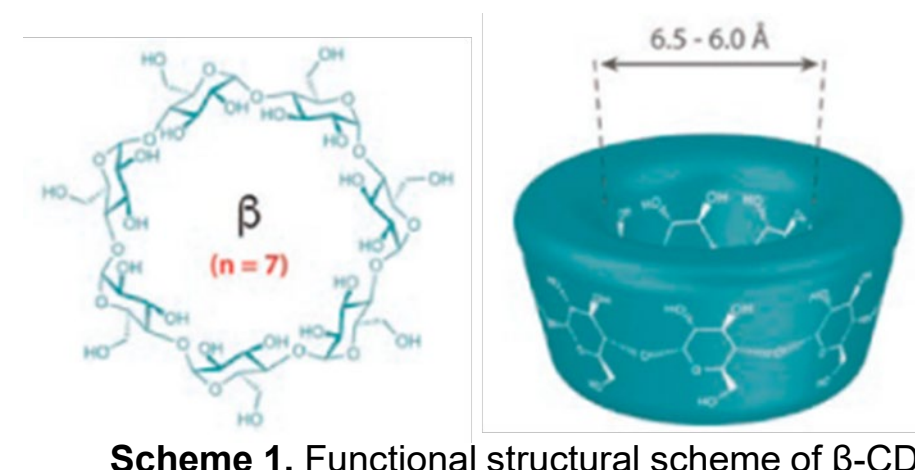
Oregano essential oil

OBJECTIVES:

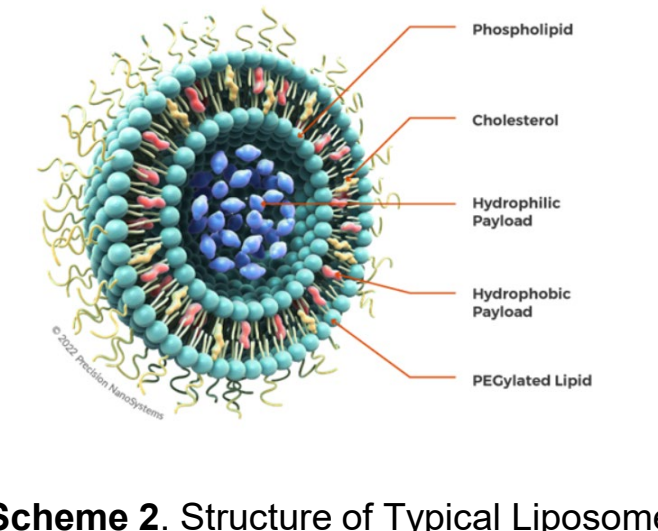
To study the antimicrobial activity against *L. monocytogenes* and *Salmonella* spp. of encapsulated OEO in two different inclusion complexes (β -CDs and liposomes), compared to free OEO in Kefir milk and Katiki Domokou cream cheese.

A. Encapsulation of OEO

The encapsulation of OEO in β -CD (**Scheme 1**) was performed with the co-precipitation method (Garcia-Sotero et al., 2019) in two different ratios of OEO: β -CDs (1:99 and 8:92), whereas for the incorporation of OEO into liposomes (**Scheme 2**) the thin-film hydration method was applied (Moghimpour et al., 2012).



Scheme 1. Functional structural scheme of β -CD.



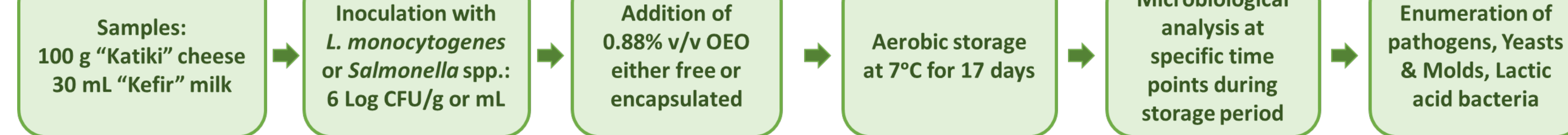
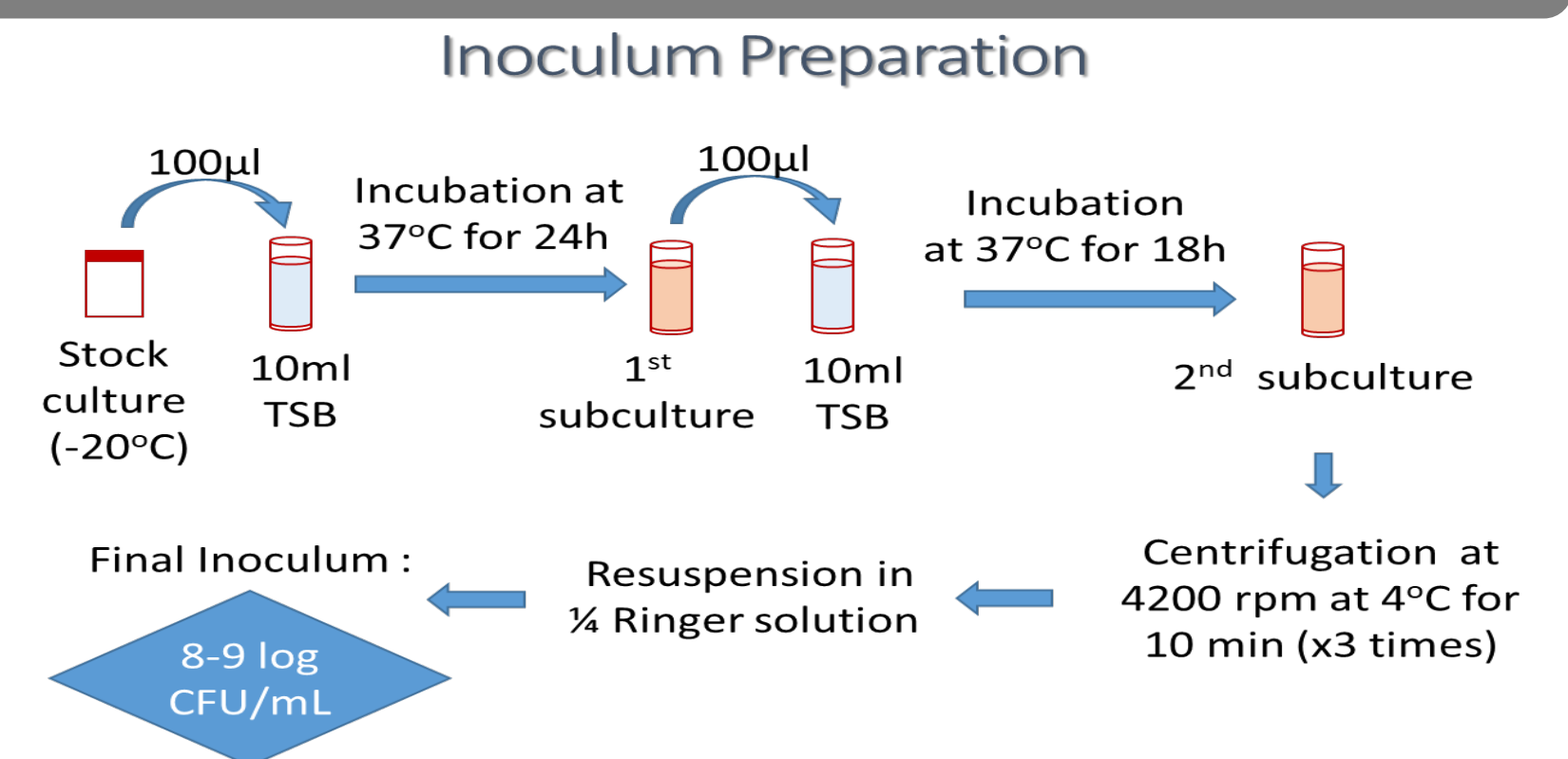
Scheme 2. Structure of Typical Liposome

B. In situ application

Nutrition Information (per 100g or ml)	“Katiki” cheese	“Kefir” milk
Total Fat	13 g	1.5 g
of which saturated	8 g	0.9 g
Carbohydrates	3 g	3.6 g
of which sugars	3 g	3.6 g
Protein	10 g	3.3 g
Salt	1 g	0.11 g



Bacterial strains
<i>Listeria monocytogenes</i> C5
<i>Listeria monocytogenes</i> 6179
<i>Listeria monocytogenes</i> ScottA
<i>S. Typhimurium</i> 4/74
<i>S. Typhimurium</i> DT 193
<i>S. Infantis</i> 167



**OEO: β -CDs at 1:99 ratio could not be applied to kefir milk because changed its physicochemical characteristics

➤ pH and a_w were also measured on the first and the last day of the storage period

Microorganism	Media	Incubation
<i>L. monocytogenes</i>	ALOA	37°C / 2 days
<i>Salmonella</i> spp.	XLD	37°C / 1 day
Yeasts	RBC	25°C / 3-5 days
Lactic acid bacteria	MRS	30°C / 3 days

RESULTS

KATIKI CHEESE

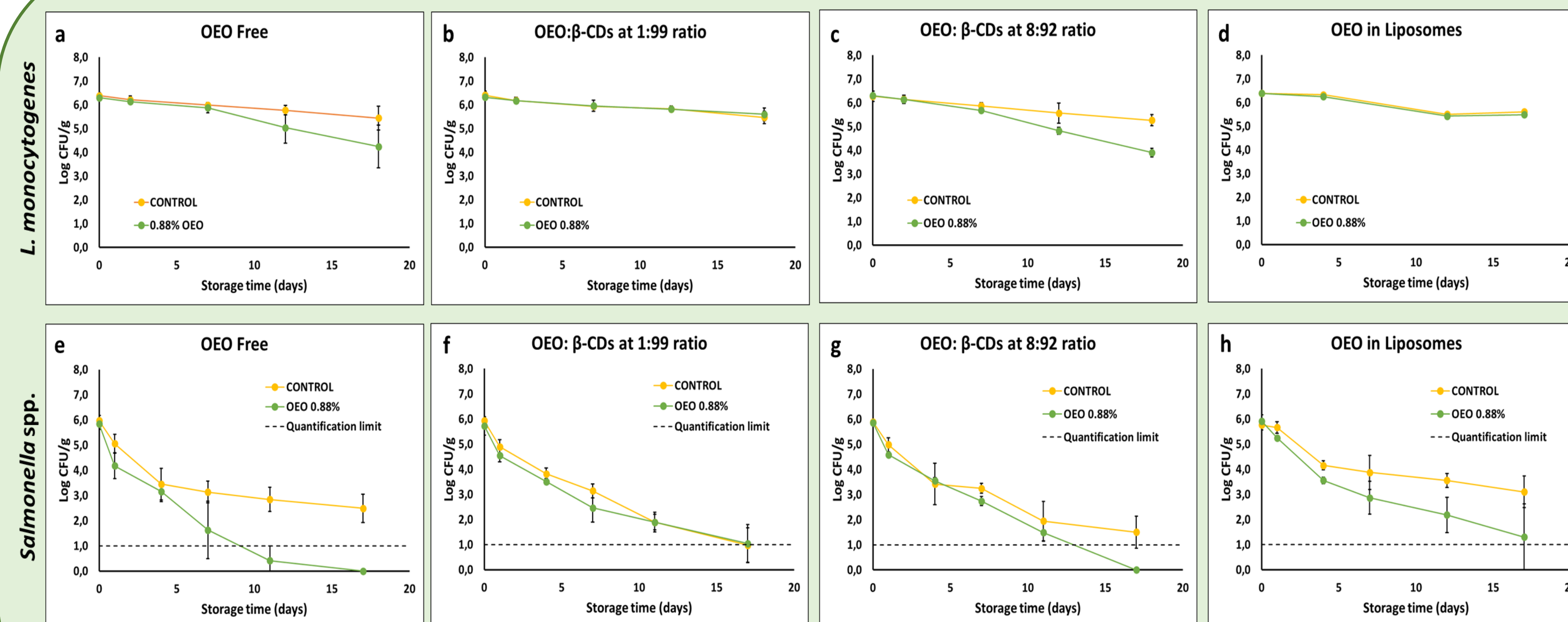


Figure 1: Survival curves (mean values $\pm$ standard deviations, n=4) of *Listeria monocytogenes* (a, b, c, d) and *Salmonella* spp. (e, f, g, h) cocktail strains in “Katiki” cheese containing 0.88% (v/v) of free and encapsulated OEO during aerobic storage at 7 °C

“KEFIR” MILK

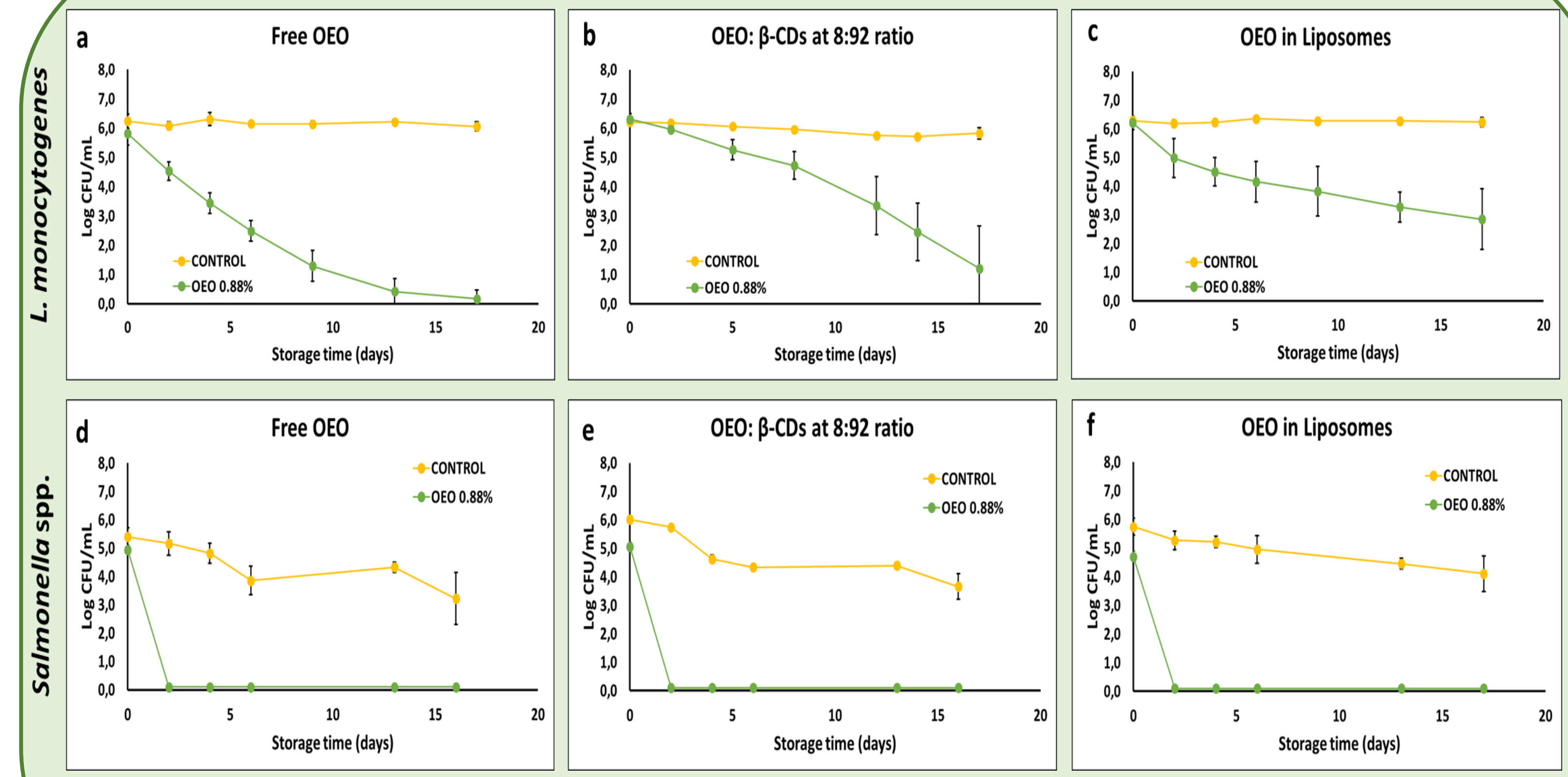
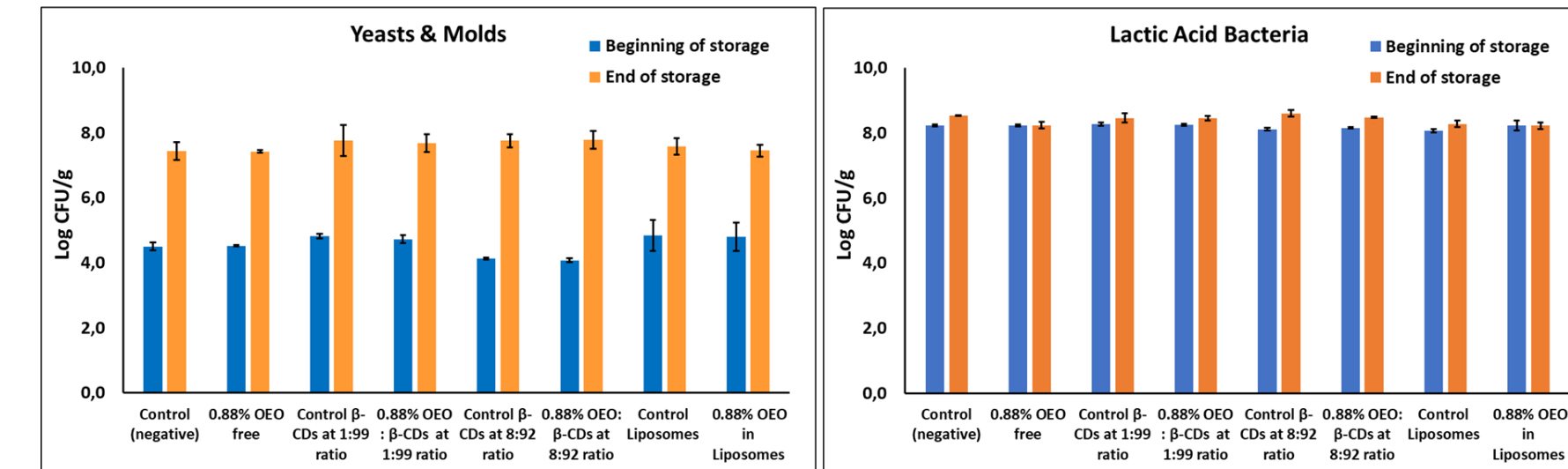


Figure 2: Survival curves (mean values $\pm$ standard deviations, n=4) of *Listeria monocytogenes* (a, b, c) and *Salmonella* spp. (d, e, f) cocktail strains in “Kefir” milk containing 0.88% (v/v) of free and encapsulated OEO during aerobic storage at 7 °C

(i) KATIKI CHEESE



(ii) KEFIR MILK

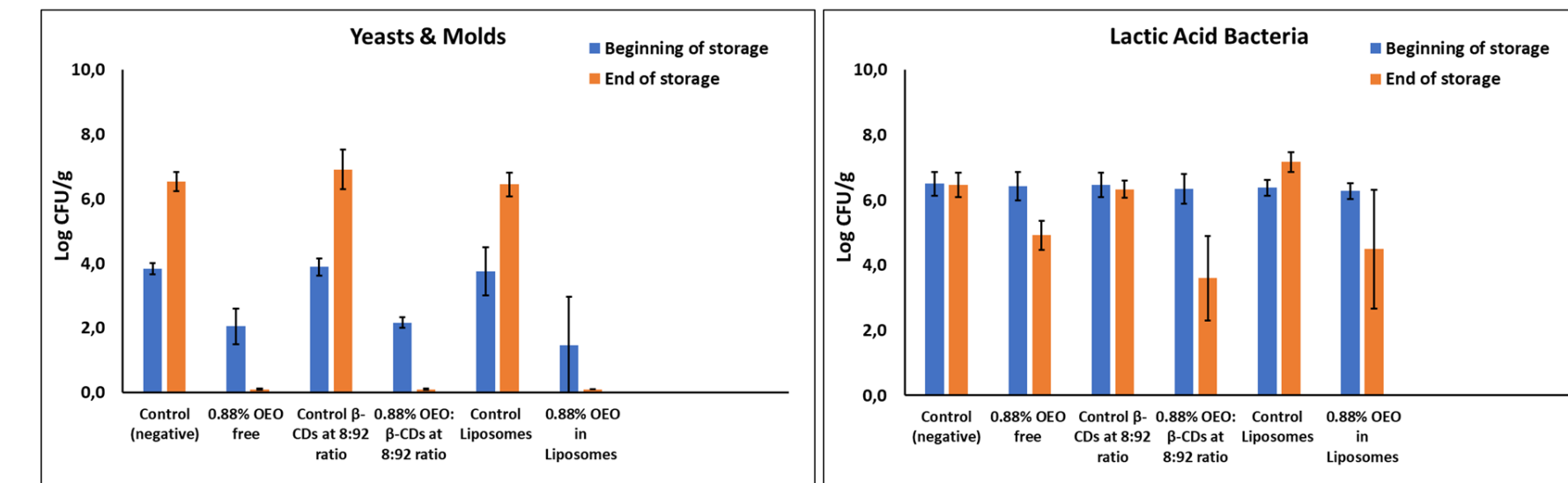


Figure 3: Populations of Yeasts & Molds and Lactic Acid Bacteria (mean values $\pm$ standard deviations, n=4) in (i) katiki cheese and (ii) Kefir milk containing 0.88% (v/v) of free and encapsulated OEO during aerobic storage at 7°C.

➤ Regardless of the food matrix (milk vs soft cheese), *Salmonella* spp. appeared to be more sensitive compared to *L. monocytogenes* (Fig. 1 & 2).

➤ In “Katiki” cheese:

❑ OEO at 0.88% (v/v) when applied against *L. monocytogenes* exhibited the following order of inactivation: Free OEO at 0.88% > OEO at 0.88% in β -CDs at the 8: 92 ratio >> OEO at 0.88% in Liposomes (Fig. 1).

❑ OEO at 0.88% (v/v) when applied against *Salmonella* spp. exhibited the following order of inactivation: Free OEO at 0.88% > OEO at 0.88% in β -CDs at the 8: 92 ratio > OEO at 0.88% in β -CDs at the 1: 99 ratio > Liposomes (Fig. 1).

➤ In “Kefir” milk

❑ Free OEO at 0.88% (v/v) resulted in almost 6 Log reductions of *L. monocytogenes*. OEO at 0.88% (v/v) when encapsulated into β -CDs at the 8: 92 ratio and into liposomes caused 5.1 and 3.4 log reduction, respectively (Figure 2).

➤ OEO (free or encapsulated) reduced the population of yeasts and lactic acid bacteria in “kefir milk” (Fig. 3 ii) while had no effect on the microbiota of “katiki” cheese (Fig. 3 i).

➤ Addition of OEO (free or encapsulated in β -CDs) did not affect the physicochemical characteristics (pH & a_w) of the products (Fig. 4). Addition of liposomes increased the pH & decreased the a_w of Kefir milk (Fig. 4ib).

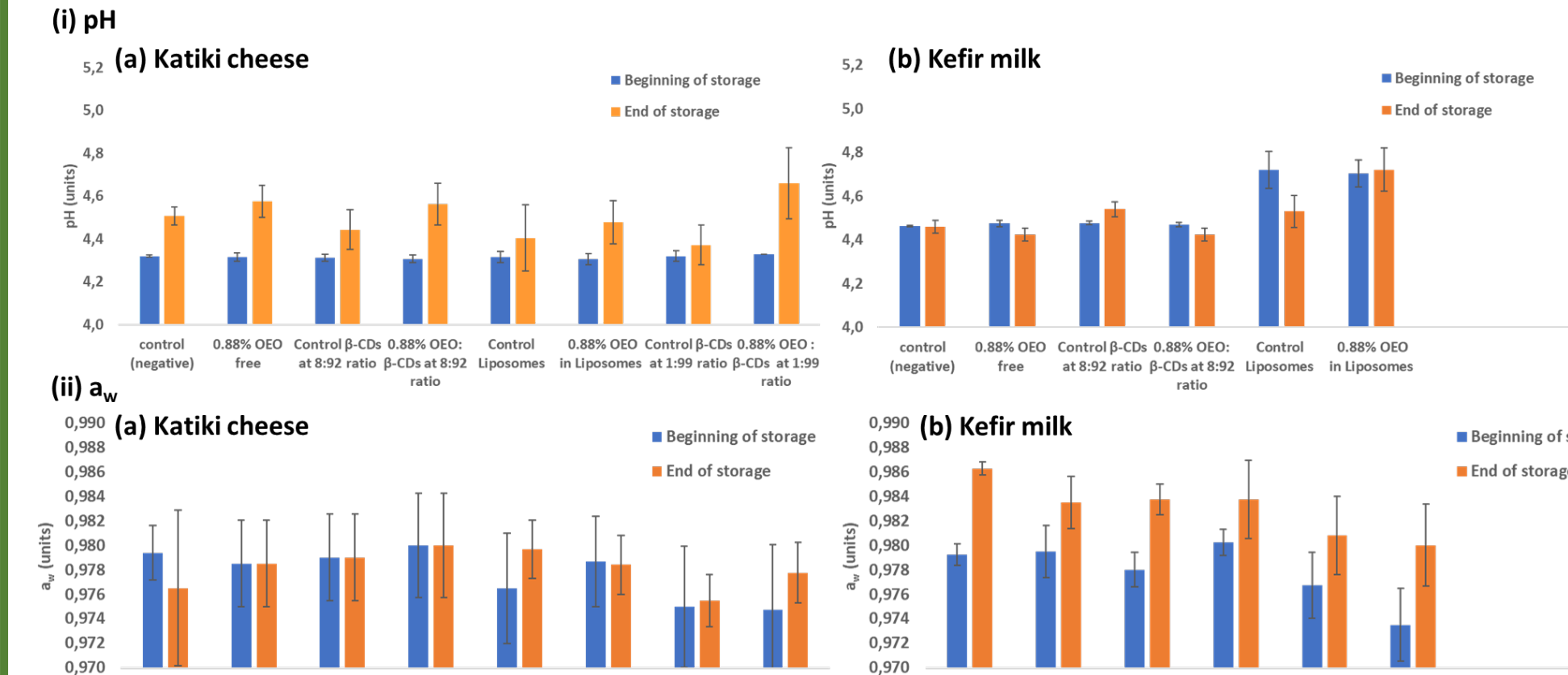


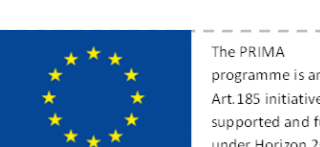
Figure 4: Changes in (i) pH and (ii) a_w of (a) “Katiki” cheese and (b) kefir milk containing 0.88% (v/v) of free and encapsulated OEO during aerobic storage at 7°C.

CONCLUSIONS

▪ Encapsulation of OEO into different inclusion complexes exhibited different antimicrobial activity which may also be affected by food matrix and microorganisms.

REFERENCES:
Garcia-Sotelo et al., (2019), Antimicrobial activity and thermal stability of rosemary essential oil: β -cyclodextrin capsules applied in tomato juice. LWT - Food Science and Technology (111), 837-845.
Moghimpour et al., (2012), Preparation and Characterization of Liposomes Containing Essential Oil of *Eucalyptus camaldulensis* Leaf. Jundishapur J Nat Pharm Prod. (7), 117-22.

Acknowledgments:



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