

Optimization of linoleic acid emulsion preparation to reduce substrate losses after filter-sterilization

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Introduction

Probiotic strains have demonstrated the capacity to convert linoleic acid (LA) into conjugated linoleic acid (CLA) isomers, a group of conjugated fatty acids well-characterized for its bioactive properties¹.

In vitro studies focused on CLA microbial production normally test the capacity of potential CLA-producing strains by culturing them in the presence of the precursor substrate, which in this case is LA². In most assays the LA is added as a stock solution prepared with pure LA and an emulsifier, usually Tween 80, at specific concentrations, dissolved in distilled water. Afterwards the mixture is sterilized through filtration^{3,4}. However, this preparation procedure leads to LA losses requiring higher amounts of LA to achieve the intended concentration.

Objectives

The main aim of this work was to optimize the LA emulsification strategy in order to obtain a more efficient and cost-effective procedure.

To do so the following specific objectives were pursued:

- Test the impact of treating LA emulsion with Ultra-Turrax prior to filtration;
- Test the impact of treating LA emulsion with Sonicator prior to filtration;
- Test the profitability of the more suitable treatment procedure when applying a smaller pore sized membrane that assures microbiological sterility.

Methods

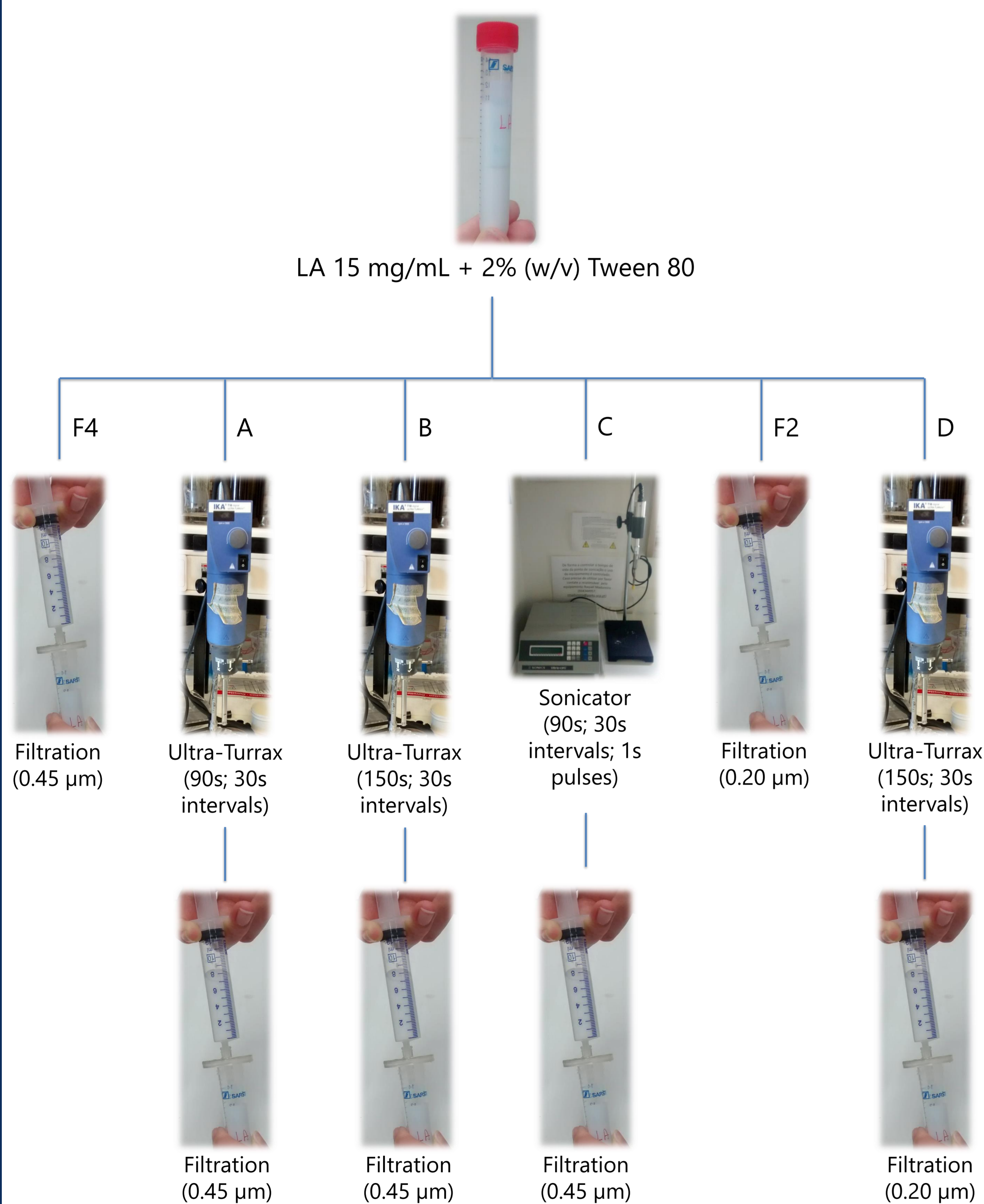


Figure 1. Experimental design

Results

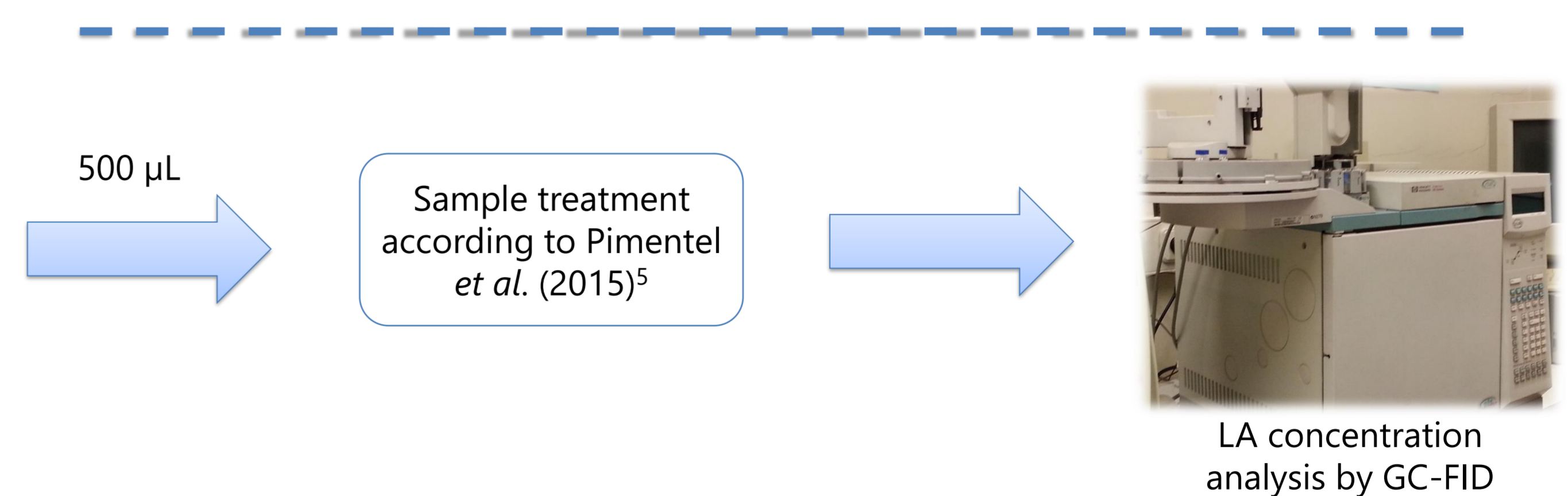
Filtration of LA emulsion directly through a 0.45 µm-pore size membrane (F4) led to a 17.09% LA loss whereas the introduction of a blending step, either using a sonicator (C) or an Ultra-Turrax (A and B) led to lower losses of 13.01%, 7.17% and 7.40%, respectively (Table 1).

Since there were no significant differences between treatments A and B ($p > 0.05$) with Ultra-Turrax, which demonstrated the lowest LA losses, treatment B was chosen to be further tested with a smaller pore size filter of 0.20 µm (D). The application of a smaller pore size membrane also contributed to lower losses; LA reductions were from 10.07% (F2) to 3.71% (D) (Table 1).

Table 1. LA losses after filtration (F4, F2), Ultra-Turrax (A, B, D) and sonicator (C) treatments with 0.45 and 0.20 µm-pore size membranes.

	0.45 µm-pore size			
	Filtration (F4)	Ultra-Turrax (A)	Ultra-Turrax (B)	Sonicator (C)
LA loss (%)*	17.09 ± 1.47	7.17 ± 2.87	7.40 ± 2.82	13.01 ± 1.34
	0.20 µm-pore size			
	Filtration (F2)	Ultra-Turrax (D)		
LA loss (%)*	10.07 ± 0.05	3.71 ± 1.28		

*Mean values ± standard deviation (n=2).



Conclusions

In conclusion, a previous dispersion with Ultra-Turrax, independent of the filter pore size, demonstrated to be the best method to reduce substrate losses in filter-sterilization of LA emulsions. This brings new insights for more cost-effective CLA producing assays.

References

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Acknowledgements

This work was financed by National funds via FCT – Fundação para a Ciência e Tecnologia, under the project “Pro-TECh-CLnA - Microbial Production of Bioactive Conjugated Linolenic Acid Isomers to Obtain Functional Ingredients and Foods” reference PTDC/AGR-TEC/2125/2014. Financial support for the authors A.L. Fontes and L.L. Pimentel was provided by fellowships SFRH/BD/117721/2016 and SFRH/BPD/119785/2016, respectively, granted by the Portuguese government through FCT.

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