# Optimized Production of Human Milk Fat Substitutes by Acidolysis of Lard and Fatty Acids from a Fish Oil Concentrate Catalysed by a Heterologous *Rhizopus oryzae* Lipase

Tiago Simões[1], Marina Guillén[2], Francisco Valero[2],
Carla Tecelão[1,3], Suzana Ferreira-Dias[3]

[1]Marine Resources Research Group, School of Tourism and Maritime Technology, Polytechnic Institute of Leiria, P-2520-641, Peniche, Portugal.
[2]Departament d'Enginyeria Quimica. (EE). Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain
[3]Instituto Superior de Agronomia, CEER-Biosystems Engineering, Technical University of Lisbon, Tapada da Ajuda, Lisbon, Portugal
E-mail: carlatecelao@ipleiria.pt

In human milk fat (HMF), palmitic acid, the major saturated fatty acid, is mostly esterified at the internal position of the triacylglycerols (TAG), while the unsaturated fatty acids occupy the external positions. This study aims at the production of HMF substitutes (HMFS) by acidolysis reaction between lard and the free fatty acids from a fish oil concentrate rich in docosahexaenoic acid, in solvent-free media. The immobilized commercial lipases from (i) *Rhizomucor miehei* (Lipozyme RM IM), (ii) *Thermomyces lanuginosa* (Lipozyme TL IM), (iii) *Candida antarctica* (Novozym 435) and (iv) the non-immobilized lipase from *Pseudomonas fluorescens* (Amano AK) were tested as biocatalyst. Also, the heterologous *Rhizopus oryzae* lipase (*r*ROL), immobilized in Accurel® MP 1000, was tested as a feasible alternative to the commercial lipases. After 24h reaction at 50ºC, polyunsaturated fatty acid (PUFA) molar incorporations of 17.5% for "Novozym 435", 16.9% for *r*ROL, 16.8% for "Lipozyme RM IM" and 4.4% for Amano AK were attained. Modeling acidolysis catalyzed by rROL and optimization of reaction conditions were performed by response surface methodology, as a function of molar ratio and temperature. The highest acidolysis activity was achieved at 40ºC at a molar ratio of 1:3 (lard/PUFA), decreasing with both temperature and molar ratio. Operational stability studies for *r*ROL in consecutive 24h batches were carried out. After 3 reuses, the biocatalyst retained about 55% of the original activity.