OPTIMIZATION OF MEDIUM CHAIN LENGTH FATTY ACID INCORPORATION INTO OLIVE OIL CATALYZED BY IMMOBILIZED LIP2 FROM *Yarrowia lipolytica*

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Introduction. Structured lipids (SL) are triacylglycerols (TAG) that have been restructured to change the fatty acids positions in the glycerol backbone, modified by the incorporation of new fatty acids or synthesized de novo to yield novel TAG, either chemically or enzymatically (1). MLM type lipids are SL with medium chain length fatty acids (M), containing between 6 and 10 carbons, in the sn-1 and sn-3 positions, and long chain fatty acids (L), with more than 12 carbons, at the sn-2 position. This type of SL avoid have targeted nutritional, energetic and pharmaceutical properties (2).
The objective was the production of SL by enzymatic acidolysis between virgin olive oil and caprylic or capric acids using Lip2 from Yarrowia lipolytica. Lip2 from *Y. lipolytica* immobilized on Accurel MP 1000 (YLL2) was tested in a solvent-free system. In addition, the reactions were optimized by response surface methodology (RSM).

Methods. The acidolysis reaction consisted of 3g of virgin olive oil (OO) and different amounts of caprylic (C8:0) or capric acid (C10:0) corresponding to molar ratios of FFA/TAG of 1:1 to 8:1. The immobilized lipase amount used was fixed (5 wt% of total substrates) and different temperatures (30-50°C) were tested. Reactions were carried out in solvent-free system in thermostated-capped cylindrical glass tubes under magnetic agitation.
Response Surface Methodology (RSM) (3) was used to model the acidolysis of virgin olive oil with C8:0 and C10:0 and to optimize the reaction conditions with YLL2. For optimization with C8:0, 17 experiments (3 central points, 8 factorial points and 6 stars points) were carried out following the central composite rotatable design (CCRD), as a function of molar ratio (MR), temperature (T) and reaction time (t). Optimization with C10:0 was carried out with a total of 11 experiments (3 central points, 4 factorial points and 4 stars points) following the CCRD as a function of MR and reaction time.

Results. Incorporation values of C8:0 or C10:0 into virgin olive oil, by acidolysis reaction, in solvent-free media, catalyzed by immobilized YLL2, under the conditions of the experimental designs followed, are presented in Fig. 1 in the form of response surfaces. C8:0 incorporation into olive oil can be fitted to a flat response surface (Fig.1, A), described by a first-order polynomial equation (Table 1). C10:0 incorporation (Fig.1, B) can be described by a second-order polynomial equation (Table 1).

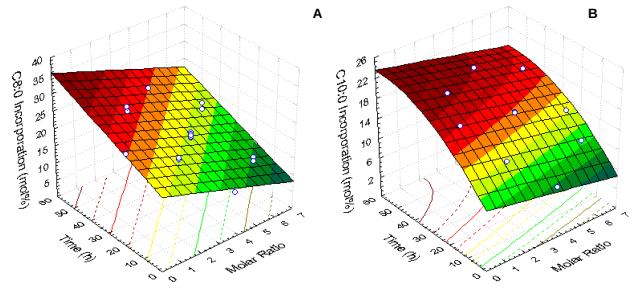

Fig.1 Response surface fitted to the incorporation of C8:0 (A) or C10:0 (B) into virgin olive oil by acidolysis with YLL2, as a function of reaction time and molar ratio FFA/TAG

Table 1. Model equations of the response surface fitted to the acidolysis of olive oil with caprylic or capric acid catalyzed by YLL2 as a function of molar ratio FFA/TAG (MR) and reaction time (t, h).

System	Model Equation	R^2
OO + C8:0	$C8:0_{\%mol\ incorporation} = 17{,}41 - 2{,}23 \cdot MR + 0{,}289 \cdot t$	0.89
OO + C10:0	$C10:0_{\%mol\ incorporation} = 6{,}91 - 0{,}92 \cdot MR + 0{,}55 \cdot t - 0{,}0047 \cdot t^2$	0.93

From the response-surfaces no optimal points were observed inside the considered experimental region, only the identification of the regions corresponding to higher incorporations could be achieved. For both systems, the highest incorporations inside the experimental domain should be reached at 40°C, with a molar ratio of 2:1 FFA/TAG and a reaction time of 48h. Under optimized conditions the highest incorporation was reached for C8:0 (25.6%mol) and C10:0 (21.3%mol).

Conclusions. Production of SL from olive oil and medium chain fatty acids was successfully achieved with Lip2 from *Y. lipolytica* immobilized in Accurel MP 1000. The maximal incorporation obtained was of 25.6% for C8:0 and of 21.3% for C10:0.

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