Hydrolysis of Milk Fat Globules Catalyzed by Lipases with Distinct Specificities: Kinetics and Structural Modifications

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Lipids are present in their native state as dispersed droplets in milk plasma, called milk fat globules (average ø 4 µm). The globules are heterogeneous: their triacylglycerol core (98 % w/w) is enveloped by a trilayered biological membrane mainly based on polar lipids and glycosylated proteins. This emulsion presents a large interfacial surface and is submitted naturally to the action of various endo- or exogenous lipolytic enzymes during milk storage and transformation. However, the kinetics and mechanisms of hydrolysis of the milk fat globules catalyzed *in vitro* by lipases with diverse specificities are not well-known. The objective of the present study was to determine the kinetics and mechanisms of hydrolysis of native globules subjected to the action of a *sn*-1,3 specific lipase (*M. miehei*) or to the action of a non specific lipase (*C. rugosa*).

The kinetics of hydrolysis were monitored using pH-stat method. To explain the kinetics, the emulsion was also characterized at molecular level (evolution of classes of lipids) and macromolecular level (apparent surface charge, particles size distribution, particles shape).

Our results indicated that i) the *sn*-1,3 specific lipase induced a Mickaelian shape without time lag caused by the presence of the globule membrane and an important retro-inhibition after 5 % hydrolysis of the acyl moieties; ii) the non-specific lipase induced a sigmoidal shape with a time lag due to the presence of membrane constituents. The *sn*-1,3 lipase triggered mainly partial aggregation and vesicularisation of particles whereas the non-specific lipase triggered more coalescence. Enzyme concentration, temperature and or pretreatment of globule substrate with phospholipase or protease were key parameters that affected the observed kinetics for both lipases. These results highlight the possibility of modifying macromolecular and interfacial organization of milk fat globule by playing on lipolytic enzymes specificity.

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