## Abstract

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The outermost layer of the epidermis, the *stratum corneum*, is where the skin barrier is located, consisting of corneocytes surrounded by an organized lipid matrix. Maintaining the protective function of the skin depends on the lamellar and lateral organization of barrier lipids in their optimal ratio. A key factor influencing this organization is the pH of the environment, ideally around 5.5. Disruption of this balance can lead to impaired barrier function and the development of skin diseases. Therefore, understanding these processes is crucial for the development of more effective therapeutic approaches. The aim of this study was to develop a validated and reproducible experimental model to investigate the effect of pH on the structure of the skin barrier. Aqueous dispersions of isolated human epidermal lipids (5 mg/mL) were acidified using hydrochloric and acetic acids at various concentrations to reach a pH of near 5.5. The effects of both direct acidification and gradual acidification via a dialysis membrane were compared. Changes in lamellar structure (short- and long-periodicity phases) and the lateral packing of lipids (orthorhombic and hexagonal phases) were analysed using X- ray diffraction and Fourier-transform infrared spectroscopy, providing detailed insights into the molecular arrangement of the lipids.