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Fluorescence Lifetime pH Measurements in Cheese Matrix

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The pH of cheese affects the texture of curd directly by influencing the hydratation of the caseins; all else being equal, high pH cheeses are softer than more acid cheeses. pH also affects texture and flavour indirectly by affecting the activity of enzymes important to ripening and, in the case of the coagulant, the retention of enzyme in the curd during manufacture [1]. Since low pH is known to modify the metabolic activity of lactic acid bacteria and to have even more influence when then cells are immobilized it is important to determine local pH in such a complex solid structure such as cheese [2].

We monitored the pH both at the macroscopic scale (macro pH) and at the microscopic scale, using a non destructive microscopic techniques employing C-SNARF-4 fluorescent probe. When excited between 488–561 nm the dye exhibits two emission peaks (around 580 nm and 640 nm) whose intensities display different pH dependencies. We have confirmed the results of [3] that the fluorescence ratio I_{580nm}/I_{640nm} is a reliable indicator of pH at values 5.3 and above. In more acidic environments the fluorescence ratio is insensitive of pH (see Figure 1a).

To enable pH measurements in the range 4.5 to 5.5 we explored the fluorescent lifetimes of C-SNARF-4 in two spectral bands (‘green’, 540 – 600 nm and ‘red’, 610 – 670 nm) on a Leica SP8 AOBS confocal microscope equipped with pulsed White Light Laser and time-gated hybrid detectors. Combination of White Light Laser and time-gated hybrid detectors enables setting different time detection windows for spectral detectors. Two-component fits for dye solutions buffered to different pHs revealed a clear dependence of the longer lifetime component of the red spectral band on pH in the range 4.5 to 6.5 (see Figure 1a).

To further explore the fluorescence properties of C-SNARF-4 and their potential for pH measurements we performed excitation-emission lambda scans on buffered solutions of the dye (see Figure 2). The results indicate that the pH sensitivity of the probe can be enhanced by proper choice of excitation wavelength combined with lifetime measurements of the 640 nm spectral component of the emission.

We have also acquired FLIM images of various types of cheese using Leica SP8 X with time correlated single photon counting detection by internal spectral FLIM dedicated PMTs. An excitation laser line out of White Light Laser was 555 nm with 80 MHz repetition rate and the detection was performed by PicoQuant TCSPC equipment. As the fluorescence lifetime is an intrinsic property of given dye and does not depend on instrumentation, we could use our calibration results to map the observed lifetime to a local pH information (see Figure 1b). Results indicate for the first time that localised pH measurement can be accomplished in complex foods using FLIM [4].

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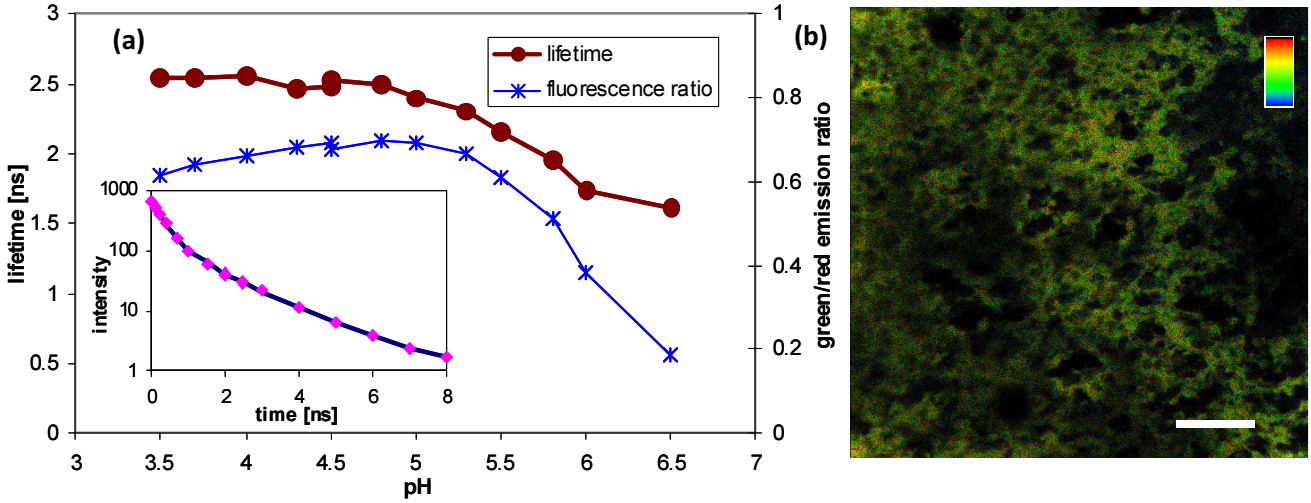

Figure 1. (a) The pH dependence of C-SNARF-4 fluorescence: lifetime of the longer component in the 'red' emission band and the ratio of fluorescence intensities in the 'red' and 'green' emission bans. Inset shows a typical fluorescence decay curve with (symbols) a two-component fit (line). The detectors were gated with a 3.5 ns window with a delay of 0.1 to 8 ns after the excitation pulse. **(b)** FLIM images of cheese matrix acquired by Leica TCS SP8 SMD microscope with TCSPC FLIM equipment, scale bar = 100 μm. False colors represent local lifetimes from 0 ns (blue) to 7 ns (red).

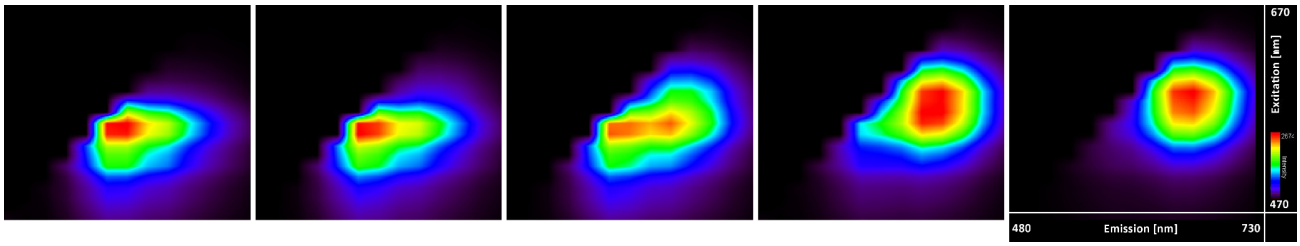

Figure 2. Excitation-emission lambda scans of the C-SNARF-4 solution buffered to different pH. Vertical axis shows excitation wavelengths of White Light Laser (470-670 nm), whereas horizontal axis displays emission spectra (480-730 nm) of C-SNARF-4 in different pH from left: 4.5, 5.0. 5.5, 6.0 and 6.5. Increase of pH value results in moving maximum emission from the blue part of spectra towards the red part of spectra with forming two peaks horizontally for pH 5.5 and two peaks vertically for pH 6.0.

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