

Cooking Eggs in NMR Measuring the Denaturation of Egg Protein using T1&T2 Relaxometry

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<u>Introduction:</u> Protein denaturation is a fundamental process in food science and biochemistry, involving structural changes in protein molecules under thermal stress [1]. Chicken eggs, which contain a variety of proteins with distinct thermal stabilities, serve as an excellent model system for investigating heat-induced denaturation. Traditional analytical methods often lack the temporal resolution or sensitivity to capture real-time molecular transitions. Time-Domain Nuclear Magnetic Resonance (TD-NMR) offers a non-invasive and sensitive approach to monitoring these structural changes via T1 and T2 relaxation times, which reflect molecular mobility and interactions in the protein matrix [2]. In this study, TD-NMR with controlled temperature variation was used to monitor the denaturation behavior of egg proteins during the cooking process.

Methods: Fresh chicken eggs were separated into egg white and egg yolk, and samples were prepared in 15 mm NMR tubes. Additionally, a mixed sample of egg white and yolk was created, and vegetable oil was used as a reference. Samples were placed in a variable-temperature (VT) NMR probe and initially equilibrated at 49 °C. The temperature was then increased in 1 °C steps up to 90 °C, with measurements taken at each step after 3 minutes of thermal equilibration. Subsequently, the temperature was decreased in the same manner. For each temperature point, T1 inversion recovery and T2 Carr-Purcell-Meiboom-Gill (CPMG) measurements were performed. The resulting relaxation data were fitted using both single- and double-exponential models. For analysis, T1 and T2 values and fitted signal amplitudes at time t = 0 were used.

Results: Figure 1 presents the estimated fraction of nondenatured proteins as a function of temperature, with the total denatured content shown in red and the T2 trend of egg white shown in purple. The observed T2 changes correlate with the known denaturation temperatures specific proteins, particularly Ovalbumin and Ovomucoid.

Fig. 1: left: Egg white, yoke and mixed egg in glass tubes after denaturation experiment. Right: Fraction of the non-denaturated proteins compared to T2 values.

<u>Conclusion:</u> Both the relaxation times and the extrapolated signal

amplitudes at t = 0 provide valuable insights into the thermal denaturation behavior of egg proteins. These changes can be linked to the known thermal stability of specific proteins, demonstrating the potential of TD-NMR relaxometry for real-time, non-invasive monitoring of protein structure during cooking processes.

<u>References:</u> [1] Y. Mine, Trends in Food Science&Technology, 6(7):225-32, 1995. [2] J.van Duynhoven et al., Ann Rep on NMR Spectroscopy, 69:145-97, 2010.