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Confocal Raman microspectroscopy study of vitelline membranes from hen eggs

Context and Objective: The vitelline membrane (VM) surrounds the yolk of the hen's egg and separates it from the albumen. It consists of two major layers, the inner layer, which is laid down in the ovary, and the outer layer, which is secreted in the oviducts. VM is known to stretch and become weakened with decrease of the egg quality (1). In order to get a deeper insight into VM structure and role it plays in the hen egg quality and preservation against bacteria, we investigated the effect on the molecular composition of VM induced by a series of factors such as the hen age, the egg storage duration and conditions (temperature and atmosphere). **Methods:** Lohmann Tradition laying hens were set up at the PEAT unit (INRAE Nouzilly) and reared on the ground for egg production. The eggs were collected at different ages of hens: 32, 73, and 81 weeks. The eggs were stored for 0, 14 and 21 days under controlled conditions, variable in terms of temperature (4 or 20°C), relative humidity (50 or 75-80%) and CO₂ (0 or 10%). The samples of VMs were extracted from the eggs, washed in cold water, disposed on CaF₂ substrates and dried at ambient conditions. Raman spectra were collected as hyperspectral maps (min 25 spectra each, up to 4 maps per sample, up to 2 maps on each side of the membrane) via an x50 objective of a confocal Raman microspectrometer (LabRam, Horiba Scientific, France) equipped with the 690 nm laser source. The data acquisition was made using a LabSpec software (Horiba Scientific, France). The data have been then pre-processed (EMSC, area normalization) and analyzed by ASCA (ANOVA Simultaneous Component Analysis) using MATLAB® (Mathworks, USA).

Results and Conclusions: For each sample of VM, selected according to chicken age and storage conditions (duration, temperature, and atmosphere composition), average Raman spectra were generated and compared. The obtained results (Fig 1) confirmed that confocal Raman microspectrometry is potent enough to reveal the specific features of protein composition/conformation of the VMs according to the hen's age.

References

- [1] JL Heath. Factors Affecting the Vitelline Membrane of the Hen's Egg. *POULTRY SCIENCE* 55: 936-942, 1976.
- [2] M Bregeon, N Guyot, S Réhault-Godbert. Mechanical Separation and Protein Solubilization of the Outer and Inner Perivitelline Sublayers from Hen's Eggs. *J Vis Exp* 27: 167, 2021.

Figures

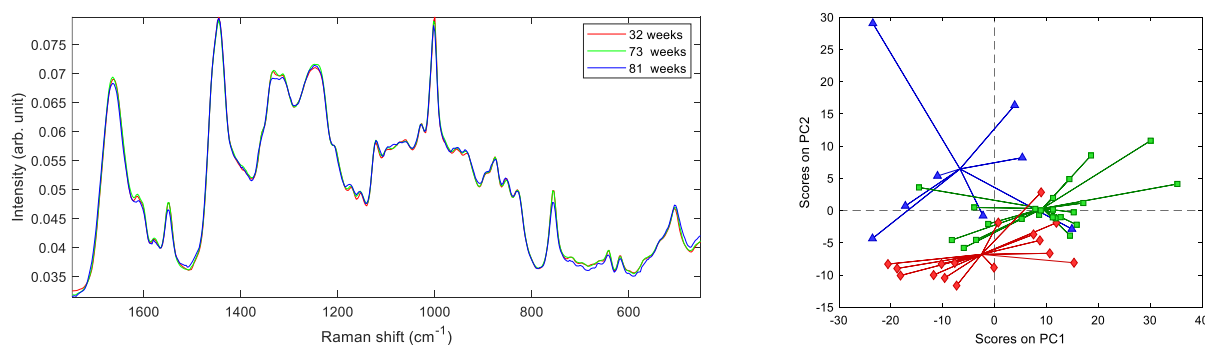


Figure 1: Distinguishing molecular changes of vitelline membranes from Raman spectra. A: Average Raman spectra of 3 groups of samples according to hen's age (Day 0). B: ASCA scores evolution for the same groups of samples.